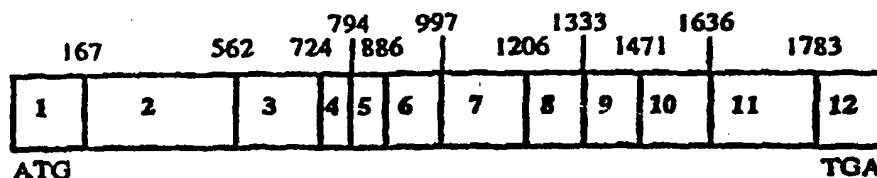




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, C07K 14/47, 16/18, C12Q 1/68, A61K 38/17		A2	(11) International Publication Number: WO 99/51740
			(43) International Publication Date: 14 October 1999 (14.10.99)
(21) International Application Number: PCT/EP99/02295 (22) International Filing Date: 2 April 1999 (02.04.99) (30) Priority Data: 98400823.5 6 April 1998 (06.04.98) EP (71) Applicants (for all designated States except US): JANSSEN PHARMACEUTICA N.V. [BE/BE]; Turnhoutseweg 30, B-2340 Beerse (BE). UNIVERSITY OF WASHINGTON [US/US]; Seattle, WA 98105-4631 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): MARTIN, Geneviève [FR/FR]; 27, rue du Lieutenant Princeteau, F-59800 Lille (FR). NEMOTO, Masami [JP/JP]; Dept. of Internal Medicine (III), Jikei University School of medicine, 1-17-1, Nishishinbashi, Minato-ku, Tokyo 105 (JP). DEEB, Samir, Sami [US/US]; 846 North East 104th Street, Seattle, WA 98125 (US). AUWERX, Johan [BE/FR]; 60, route d'Hasnon, F-59173 Millionfosse (FR). (74) Agents: ERNEST GUTMANN-YVES PLASSERAUD S.A. et al.; 3, rue Chauveau-Lagarde, F-75008 Paris (FR).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published Without international search report and to be republished upon receipt of that report.	
(54) Title: NUCLEOTIDE SEQUENCE EXPRESSING HUMAN FATTY ACID TRANSPORT PROTEIN AND CORRESPONDING AMINOACID SEQUENCE. USE FOR THE REGULATION OF FATTY ACIDS METABOLISM			



(57) Abstract

The invention relates to a nucleotide sequence which comprises a sequence involved in the expression of the human Fatty Acid Transport Protein (hFATP) comprising the aminoacid sequence of the Figure.

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Nucleotide sequence expressing human Fatty Acid Transport Protein and corresponding aminoacid sequence. Use for the regulation of fatty acids metabolism.

5 The invention relates to nucleotide sequences involved in the expression of the human Fatty Acid Transport Protein (hFATP) and relates to the Fatty Acid Transport protein.

 Fatty acids, especially long-chain fatty acids, are the structural components of several classes of lipids; they represent an important energy
10 source, particularly with respect to glucose, for various tissues or organs. Fatty acids furthermore participate in several cellular signaling processes.

 Excessive intracellular accumulation of fatty acids due either to errors in metabolisms or to increased supply can have severe pathological consequences. Furthermore, the involvement of fatty acids in several diseases
15 such as insulin resistance and coronary artery disease made the inventors focuss research efforts on the mechanisms which control the homeostasis of these lipid moieties.

 Interestingly, fatty acids are ligands and modulators of transcription factors, which control their own metabolism. This emphasizes the fact that the
20 various metabolic pathways are not only controlled by substrate supply, but also by the participation of substrates and metabolic intermediates in regulatory phenomena.

 Intracellular Fatty Acid (FA) concentrations are in part determined by proteic regulators, in particular by regulating import/export system that is
25 controlled by several proteins including Fatty Acid Transport Protein (FATP) and acyl-CoA synthetase (ACS).

 Fatty Acid Transport Protein (FATP) was first isolated in the mouse in 1994 by a functional cloning approach (Schaffer et al, 1994).

 The inventors have now isolated and characterized the human FATP
30 coding sequence and the gene comprising said coding sequence. They have furthermore identified the chromosomal location of the FATP gene, in a region

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implicated in several metabolic diseases, suggesting that FATP is part of an important group of synthetic genes. hFATP is expressed in several organs (heart, brain, liver) and tissues (adipose tissue, muscle) and is especially highly expressed in insulin-sensitive tissues. Regulatory studies suggest that its expression is under the control of several transcription factors including PPAR (Peroxisome Proliferator Activated Receptor) (Martin et al., 1997). Especially, Martin G. et al., 1997, have shown that the expression of Fatty Acid Transport Protein is regulated by PPAR α and PPAR γ activators, such as fibrates or antidiabetic thiazolidinedione. The inventors have now determined that the expression of the *hFATP* gene is also under the control of the Retinoid X Receptor (RXR) known to transduce the effects of retinoic acid on gene expression and involved in the regulation of lipid and glucose metabolism.

Therefore, FATP is an important target for therapeutic agents used in the treatment of several pathological states resulting from anomalies in regulation pathways of expression, especially of transcription or translation of the gene coding for FATP, either directly or through transcription factors.

The invention thus relates to genetic means and especially to nucleotide sequences involved in the regulation pathway of the long-chain fatty acid metabolism and therefore makes available new compounds appropriate for the definition of therapeutic means useful for treating pathological disorders related to the metabolism of long-chain fatty acids.

An object of the invention is therefore a nucleotide sequence which comprises a sequence involved in the expression, of the human Fatty Acid Transport Protein (hFATP) comprising the aminoacid sequence of Figure 2 or Figure 5.

The nucleotide sequence of the invention can be any type of sequence including any DNA especially genomic DNA, synthetic DNA, RNA and especially mRNA, said sequences being sense or antisense sequences.

Therefore, the invention relates to these sequences when they are independently of the conditions used to obtain them, thus including sequences either extracted from a biological sample, or cloned or synthesized including by

enzymatic or chemical processes.

By the expression "a sequence involved in the expression", it is intended within the present invention a sequence coding for the Fatty Acid Transport protein (FATP) and especially human FATP, or a sequence involved in the regulation of the steps required to express the FATP gene, especially the *hFATP* gene, or both sequences operably linked. Said regulation sequences can be derived or are derivable from the native genomic sequence of the FATP gene, especially *hFATP* gene.

Regarding sequence coding for the FATP, especially *hFATP*, the invention pertains to a nucleotide sequence which is capable, when placed under the control of appropriate regulation elements, especially regulation nucleotide sequences, promoter, enhancer, transcription sites, to be transcribed and under appropriate conditions to be translated into an amino acid sequence. Said aminoacid sequence can then possibly be processed, depending on the expression system chosen.

Especially, the human Fatty Acid Transport Protein which is expressed is characterized by the amino acid sequence that it comprises. Depending from the cellular host chosen for its expression, the structure of the obtained *hFATP* can vary, especially as the result of a maturation process, or of environmental conditions.

According to a specific embodiment of the invention, the nucleotide sequence comprises a sequence encoding the human FATP, corresponding to or comprising the nucleotide sequence having nucleotides 1 to 2222 of one of the sequences of figure 1, figure 2 or figure 3.

According to a preferred embodiment of the invention, the nucleotide sequence encoding the human FATP at least comprises the open-reading frame (ORF) corresponding to the human FATP represented on figure 2 or figure 5. Alternatively it comprises any fragment of said ORF, coding for a polypeptide having the functional properties of the human FATP as far as the transport of the long-chain fatty acids is concerned. The coding sequence of the human FATP gene is described on figure 1, figure 2 or figure 3.

Such a nucleotide sequence can be placed under the control of the native regulation sequences, or part thereof, present in the gene and enabling the expression of hFATP or can be placed under the control of heterologous regulation sequences according to well-known procedures.

5 According to another embodiment, the invention relates to a nucleotide sequence hybridizing in stringent conditions, with a probe comprising 50 to 2000 bp, preferably 50 to 300 nucleotides especially around 200 bp, said probe including at least 6, preferably 9 continuous nucleotides from the following sequence : CGGGGAGACGGGACGTGAAGGG.

10 As an example, a sequence which is advantageously used to provide for a probe appropriate to selectively hybridize with the nucleotide sequence of the invention comprises at least some nucleotides contained in the 5' non-coding sequence upstream from the ATG codon of the coding sequence of the gene expressing the hFATP.

15 A probe replying to this definition is considered to be specific for the human gene encoding the hFATP or derived genomic nucleotide sequences,
expressing the human FATP. Thus, it does not hybridize with murine, rat or yeast cDNA sequences. Such a probe can be an amplification product resulting from amplification with primers wherein one primer is specific for the human
20 hFATP gene, said primer comprising at least 6, preferably 9 nucleotides selected from the above nucleotide fragment.

The second primer can be taken in any region of the gene and especially in the sequence comprising nucleotides 1 to 2222 of the sequence of figure 1, or figure 3.

25 The stringent hybridization conditions used according to the invention can be defined with respect to the following parameters, referring to hybridization of a DNA probe, especially a cDNA probe with a total RNA or DNA:

- hybridization at 42°C,
- 30 - 2 washing steps at 42°C for 10 minutes in 0.5 x SSC, followed by 2 washing steps at 65°C for 30 minutes in 0.5 x SSC.

Details regarding these conditions can be found in the examples.

According to a specific embodiment of the invention, the nucleotide sequence is a genomic DNA sequence and especially is the gene encoding the human FATP.

5 According to another embodiment of the invention, the nucleotide sequence comprises a transcription initiation site 61 bp upstream from the ATG codon. Especially, the nucleotide sequence comprises the sequence coding for the hFATP and the 5' region of the gene containing regulation sequences including the transcription initiation site.

10 The structure of the sequence of the gene between the ATG and TGA codons, can be represented as follows:

794											
167		562		724		886		997		1206 1333 1471 1636 1783	
1	2	3	4	5	6	7	8	9	10	11	12
ATG										TGA	

15 The respective size of each exons illustrated on figure 8 starting from position 1 at the ATG codon is the following:

exon 1 (167 pb), exon 2 (395 pb), exon 3 (162 pb), exon 4 (70 pb), exon 5 (92 pb), exon 6 (111 pb), exon 7 (209 pb), exon 8 (127pb), exon 9 (138 pb), exon 10 (165 pb), exon 11 (147 pb), exon 12 (158 pb).

20 The introns present between said exons, have respectively the following 5' and 3' ends: intron 2 (5' gtgaggcc...gaccacag 3'); intron 3 (5' gtgagtca...tctgcag 3'); intron 4 (5' gtgagggg... cccctgcag 3'); intron 5 (5' gtactacg...ctctgcag 3'), intron 6 (...cgtcccccac 3'); intron 7 (5' gtgcacacc...cattccag 3'); intron 8 (5' gtgagcag...ctccctag 3'); intron 9 (5' gtgagcag...tctgccag 3'); intron 10 (5' gtcaagct...gcctccag 3'); intron 11 (5' gtgagcag...cactatag 3'). Intronic regions of a *hFATP* gene according to the invention are represented on figure 4 which discloses the genomic sequence encoding the human FATP.

30 Advantageously, the nucleotide sequence according to the above-given definitions comprises further sequences involved in the regulation of the

expression of the gene coding for the human FATP, present in the non coding 5' and/or 3' regions of the gene.

The sequences involved in the regulation of the expression of the gene include sequences containing the promoter region, the transcription initiation
5 site and other regions involved in the activation of the expression or in enhancement thereof.

Regulation of the expression also involves the regulation of the translation of the gene and the corresponding sequences.

Variants of the above-defined nucleotide sequences comprise a
10 sequence which either specifically hybridizes in high stringency conditions with primers having the following sequences primer 1
AAGGTCAATGAGGACACAATGG (sense), primer 2
CGAGTAGGTAGTGATCGTGACAG (antisense), or is the amplification product
obtained with the following sequences, or hybridizes in high stringency
15 conditions with said amplification product.

Another variant of the sequence is a genomic sequence coding for
hFATP and which hybridizes in high stringency conditions with a probe
containing around 200 pb, said genomic nucleotide sequence preferably
comprising a sequence involved in the regulation of the gene coding for the
20 hFATP.

A specific probe containing around 200 bp which is capable of specifically hybridizing with a genomic sequence coding for FATP can for example be found in the sequence of exon 2.

Another nucleotide sequence according to the invention is the mRNA
25 which is a sequence obtainable by transcription of a genomic nucleotide sequence coding for the hFATP and replying to the above definition.

The mRNA sequence obtainable from the gene coding for the hFATP comprises the nucleotide sequence complementary to the sequence of figure 1, or figure 3, having 2 kb. Preferably, it is a sequence including a nucleotide
30 sequence of 4.4 kb capable of hybridizing in high stringency conditions with the sequence of figure 1, figure 2 or figure 3.

The invention also specifically relates to the cDNA as obtained by reverse transcription of the mRNA defined above. Such cDNA sequence comprises the sequence of figure 1 or figure 3 containing nucleotides 1 to 2222 or the sequence of figure 2.

5 The invention also concerns polynucleotides selected among the following sequences:

- AAGGTCAATGAGGACACAATGG,
 - CGAGTAGGTAGTGATCGTGACG,
 - a sequence comprising or corresponding to sequences involved in the
- 10 regulation of the expression of the gene encoding the hFATP,
- any fragment of a nucleotide sequence defined above especially derived from the sequences disclosed in figures 1, 3 or 4, for instance by deletion mutation or insertion provided the essential biological properties of the native sequences are maintained, specific for the *hFATP* gene, including
- 15 fragments that can be used as primers for amplification reactions, or a probe for hybridization. ~~The above cited biological properties are described in the~~
examples which follow.

A further object of the invention is a vector, for the cloning or for the expression of a sequence defined above, said vector comprising, inserted in

20 site not essential for its replication, a nucleotide sequence as defined above.

Any appropriate regulation sequence, including heterologous sequences with respect to the *hFATP* gene, and especially any appropriate promoter, can be used for the expression of the nucleotide sequence of the invention cloned into the vector. The selection of these regulation sequences can depend upon

25 the cell host which is used to carry out this expression.

Especially, the vector is a plasmid or a phage. Advantageously, it further comprises a reporter sequence such as the CAT gene or the sequence of the luciferase gene.

The sequence of the reporter gene which is present in the vector can be

30 under the control of an heterologous regulator region especially under the control of the regulation sequences of the gene encoding the hFATP.

The invention also relates to recombinant cells especially procaryotic cells or eucaryotic cells and advantageously insect or mammal cells.

The invention is also directed to the human Fatty Acid Transport Protein comprising the amino acid sequence of figure 2, or figure 5 or to any
5 polypeptide fragment having the properties of FATP regarding intracellular transport of fatty acids, or to fragments recognized by antibodies directed against the human FATP.

The hFATP has a calculated molecular weight of 71 kDa and an apparent molecular weight of 63 kDa in Northern Blot analysis.

10 According to a specific embodiment of the invention, the hFATP has an isoelectric point of 8.5 and replies to the amino acid sequence of figure 2 or figure 5. According to another embodiment, this sequence is encoded by a nucleotide sequence as defined above.

Advantageously, said human hFATP is processed and especially devoid
15 of its signal peptide.

~~The hFATP of the invention can be either under its glycosylated form or~~
can be devoid of its glycosylation groups.

The invention also relates to antibodies specifically directed against hFATP.

20 In view of the various properties which have been identified with respect to the FATP in the regulation pathway of the long-chain fatty acid, the invention provides means that can be used in therapeutic compositions especially for modulation of the intracellular level of long-chain fatty acids, which comprises a component capable of regulating the expression of the gene encoding hFATP.

25 Alternatively, the composition of the invention can be used for the therapeutic modulation of the blood level of long-chain fatty acids.

The inventors have especially found out that the involvement of human FATP in the modulation of the metabolism of fatty acids, especially long-chain fatty acids, can provide a way to treat pathological states related to various
30 diseases and for example to obesity, cardiomyopathies and diabetes, especially diabetes non-insulino dependent.

According to the invention, the expression "treatment" relates to the capacity of a composition or compound, to prevent the occurrence of a pathological state or to control such pathological state or to improve the condition of a patient suffering from a pathological state, where this pathological state is the result or involves an abnormal regulation of the long-chain fatty acids metabolism.

Especially, the invention relates to such a composition which comprises an inhibitor of the expression of the FATP gene, for instance for the treatment of obesity or related diseases.

Alternatively, the invention relates to a composition which is capable of enhancing the expression of the *hFATP* gene for the treatment of cardiomyopathies, diabetes or related diseases.

The invention further provides means enabling assaying the capacity of chosen compounds to have an agonist or antagonist activity with respect to the expression of FATP, especially human FATP, in various tissues. Such assay can hence comprise the steps of:

- contacting a culture of cells of a specific tissue, said cells containing the *hFATP* gene, with a tested compound;
- detecting the effect of said compound on the expression of the FATP gene.

The invention also relates to a method for the screening of the expression of the *hFATP* protein in determined cells when these cells have been contacted with a determined compound, which method comprises the step of detecting the transcription of the mRNA in the cells or cell extracts.

In a specific embodiment of the invention, the screening method comprises

a) measuring the level of transcription of the mRNA in cells or cell extracts, wherein the cells have previously been contacted with the determined compound, in conditions enabling the interaction of said cells and said determined compound;

b) measuring the level of transcription of the mRNA in the same cells

species as in step a) or on extracts of these cells, wherein these cells have not been previously contacted with the assayed compound;

c) comparing the level of transcription obtained in steps a) and b).

Preferably the cells which are used to carry out the above screening
5 methods are selected among the group of live cells, heart cells, adipose tissue cells, skeletal muscle cells. The above defined methods can allow the identification of agonists or antagonists of the expression of the *hFATP* gene.

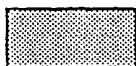
Specific elements of the invention are further disclosed in the examples and in the figures:

10 Figure 1: nucleotide sequence of clones containing 2 kb sequences obtained from a cDNA library of human adipose tissue. The sequence on the upper line corresponds to the sequence encoding the hFATP protein and the sequence on the bottom line corresponds to the alternative form of splicing found in the analyzed clones

15

Figure 2: Alignment of the nucleotide sequences of hFATP1, mFATP (MMU15976) and rFATP (RNU89529) and of the primary amino acid sequence, using the J. Hein method. The glycosylation sites are indicated by an asterisk and are boxed. The sequence of 11 amino acids common to the members of
20 the family of AMP-binding proteins is boxed on the alignments.

☐ Amino acid different from the murine sequence



Consensus sequence of 11 amino acids characterizing the family of AMP-binding proteins

25 ☐ Potential glycosylation site.

Figure 3: a variant of the nucleotide sequence of clones encoding the human FATP protein and obtained from a cDNA library of human adipose tissue.

30 Figure 4: sequence of the genomic sequence expressing the human FATP

protein, including the introns (lower case) and the exons (upper case).

Figure 5: The amino acid sequence encoded by the nucleotide sequence of figure 3 and corresponding to the human FATP protein.

5

Figure 6: Comparative analysis based on the Garnier-Robson, Chou-Fasman and Kyte-Doolittle algorithms of the murine and human FATP proteins.

Figure 7: Primer extension with the reverse GM6 primer on 10 µg of human visceral adipose tissue total RNA.

10

Figure 8: Provisional genomic structure of the hFATP gene. A. Genomic structure of hFATP with its 12 exons and 11 introns. The numbers in the boxes surrounding the exons indicate the exon numbers. The numbers above the boxes indicate the position of the introns. B. The approximate size of introns 2 to 11 (bp) is indicated between the dashes separating the two ends of the donor and of the recipient. Intron 6 was identified by sequencing the PAC clone. C. Alternative splicing leading to hFATP1 or its alternative splicing product hFATP. The relevant intron is intron 1, hFATP1 is normally spliced as in mice. The splicing of the variant of hFATP1 resumes beyond the intron in the coding sequence but changes the resading frame.

15

20

Figure 9. The 19p13 region. The arrow indicates the position of hFATP on chromosome 19, the shaded zone represents the frequency of the genes encountered in the different regions of the chromosome. The arm p of the chromosome is represented at the top. The region q is represented at the bottom.

25

Figure 10. A. Dose response of the effects of 9c-RA on FATP and ACS gene expression on FAO cells

30

Cells were kept 18 hr in serum-free medium and treated for 6 hr with increasing

concentrations of 9c-RA (10^{-8} M to 10^{-4} M). Control cells were incubated with BSA and vehicle. 30 μ g of total RNA were loaded per lane. Northern blot analysis was carried out and blots were hybridized with FATP, ACS, and actin probes. Quantification was performed with an imaging densitometer (Biorad GS-670). Values were normalized by comparison with actin control probe and results are expressed as percent of control signal, relative arbitrary unit (R.A.U.).

Figure 10.B. Dose response of the effects of 9c-RA and at-RA on FATP gene expression on Hep-G2 cells.

Cells were kept 18 hr in serum-free medium and treated for 6 hr with (10^{-8} M to 10^{-4} M) either 9c-RA and at-RA. Controls were incubated with BSA and vehicle. Northern blot analysis was carried out and blots were hybridized with an FATP and actin probe.

Figure 11. A. Dose response of 9c RA on differentiated 3T3-L1 cells.

Differentiated 3T3-L1 cells were treated 24 hr with different concentrations 9c-RA (0 - 10^{-6} M). Northern blot analysis was carried out and blots were hybridized with an FATP and ACS probe. Actin was used as control probe. Δ means differentiated.

Figure 11.B. Comparative induction of FATP and ACS in FAO and Caco2 cells. FAO(A) and CaCo2 (B) cells were serum deprived 18 hr and then treated during 6 hr with 9c-RA (10^{-6} M). Northern blot analysis was carried out as described and blots were hybridized with an FATP, ACS and actin probe.

Figure 12. Nuclear run on analysis of the effect of 9 cisRA on FAO and 3T3-L1 differentiated cells.

FAO cells were treated for 2 h with 9c-RA (10^{-6} M) after 18 h of growth in serum deprived conditions. Differentiated 3T3-L1 cells were treated during 12 h with the same concentration of 9c-RA. Transcription rates were then determined for

the FATP, ACS and GAPDH genes in from control cells (-RA) or 9c-RA treated cells (+RA). A Bluescript (BS) template was used as a control. Densitometric scanning of the results is depicted at the right panel.

5 Figures 13, 14 and 15

Dose-response of 9c-RA, at-RA and TTNPB and: in non-differentiated and differentiated 3T3-L1 cells.

Differentiated (3T3-L1 Δ) or non-differentiated (3T3-L1) cells were treated 24 hr with each of the retinoids indicated. Northern blot analysis was carried out and
10 blots were hybridized with an FATP (figure 13), ACS (figure 14) and LPL (figure 15) probe. Values were normalized by comparison with actin control probe and results are expressed in percent of control (R.A.U.).

Figure 16. Oleate uptake assay on 3T3-L1 differentiated cells.

15 Differentiated cells 3T3-L1 (3T3-L1 Δ) were treated for 24 hr with different concentrations of TTNPB (10^{-9} M to 10^{-5} M) and 14 C oleate uptake studies were
carried out. Results were normalized after protein quantification and expressed as % of control. Significant differences were indicated by an asterisk * $P < 0,05$.

20 Figure 17. Tissue expression of FATP in different human tissues. The human probe of the β -actin has enabled checking the regularity of the depots. The additional band obtained with this probe represents the actin specific of the muscle which is thus highly expressed in muscle and brain.

25 Figure 18. Expression of FATP in different human cell lines and comparison with the NCI adipose tissue: surrenal cortex carcinoma cells, THP1: monocytes, THP1 diff: THP1 differenciaded with PMA, Caco2: cells of colon adenocarcinoma, HepG2: liver hepatoma cells, Hep3B: hepatocytes, JEG-3: chorion carcinoma cells.

30

Figure 19. Distribution of FATP and LPL in muscular skeletal tissue, adipose

tissue, liver, colon, and intestine tissues in human. Sc: sub-cutaneous, Vis: visceral.

Exemple 1

5

1. MATERIALS AND METHODS

1. Materials

BRL 49653 and fenofibric acid were provided by Janssen Research Foundation, in Beerse, in Belgium, by the Fournier laboratories, in Daix, in France and by Ligand Pharmaceuticals, in San Diego, in the United States. The retinoic acids 9-cis-retinoic acid (9cRA), all-trans-retinoic acid (at-RA), were provided by Ligand Pharmaceuticals. All the other products, unless otherwise stated, were obtained from Sigma (St Louis, MO).

15 2. Human biopsies

The various omental and subcutaneous human adipose tissues were collected from normal or obese subjects during plastic or reconstructive operations. The muscular tissues were collected from patients who had undergone an operation in the hip region. They were the liver or skeleton muscle tissue. All the tissues were immediately frozen in liquid nitrogen.

3. Animals and treatments

Male rats of the Wistar strain were treated for various lengths of time with fenofibrate mixed with the feed in powdered form (weight/weight) or BRL 49653 by gavage, at the concentrations indicated. The weight of the animals and the intake of feed were recorded daily for the 7-day treatments and every two days for the 14-day treatments. The treatments with the fibrates did not cause significant changes in the quantity of feed consumed by the animals. Since each rat consumes approximately 20 grams of feed per day, the doses of 0.5, 0.05 and 0.005% (weight/weight) of fenofibrate correspond to 320, 32 and 3.2 mg/kg of body weight/day. At the end of the treatments, the rats were sacrificed by

exsanguination under ether anaesthetic. The triglyceride and cholesterol levels were measured using a colorimetric detection kit (Boehringer-Mannheim). The various tissues were collected, rinsed in 0.9% NaCl and immediately frozen in liquid nitrogen. The liver and the epididymal adipose tissue were weighed. The effects of starvation were studied on rats from which food had been withdrawn 14 hours before being sacrificed.

4. Cell culture

The cells of the different hepatic lines used were: Fa 32, a rat hepatoma cell line derived from Faza967 (Deschatrette et al., 1974), the mouse AML-12 hepatocytes (Wu et al., 1994), the human hepatoma line HepG2 and the FAO cells, a well differentiated subclone of the rat hepatoma line H4 II EC3. All the cells were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM), supplemented with 10% decomplemented foetal calf serum, except AML-12 and FAO. The AML-12 cells were maintained in DMEM/Ham's F-12 medium supplemented with insulin, transferrin and selenium (ITS, Collaborative Research), and dexamethasone (0.1 μ M). The FAO cells were cultured in DMEM/Ham's F-12 supplemented with 10% decomplemented serum, penicillin (200 IU/ml) and streptomycin (50 mg/ml).

The mouse preadipocyte cell lines ob1771 (Negrel et al., 1978) and 3T3-L1 (ATCC) were maintained in DMEM medium with 10% lipid-free and decomplemented foetal calf serum. The 3T3-L1 cells were differentiated by a two-day treatment with dexamethasone (0.1 μ M), isobutylmethylxanthine (0.25 mM) and insulin (0.4 μ M). These cells, following initiation of differentiation, were maintained for a further 8 days with insulin until their complete differentiation was obtained.

The L6 muscle cells were cultured in DMEM with 10% foetal calf serum up to confluence followed by spontaneous differentiation.

The Caco2 colon carcinoma cells (ATCC) were cultured in DMEM medium and 15% decomplemented foetal calf serum as well as antibiotics and non-essential amino acids. Above 80% confluence, the cells differentiate

spontaneously for 20 days.

5. RNA analysis

5.1. Extraction of RNA and quantitative analysis

5 The RNAs of the tissues and cells were prepared by the technique using
caesium chloride and guanidine isothiocyanate (Chomeczynski et al., 1987).
For the adipose tissue, a first step of centrifugation at 4°C of the
homogenization product was added in order to remove the lipids, which are
solidified at the surface, and to continue the extraction under the best
10 conditions. The *dot-blot* and *Northern-blot* hybridizations of the total or
messenger RNAs after separation on a poly A column (Stratagene) were
carried out as described in Auwerx et al. (Auwerx et al., 1988). Before being
deposited, the RNAs were assayed in duplicate by spectrophotometry
(Pharmacia Ultrospec 2000), the difference tolerated between the values was
15 less than 5%. The various messengers were measured using cDNA fragments
obtained by *reverse transcription*, rt-PCR or by restriction, as probes. The
probes were labelled with a *random primed labelling* kit (Boehringer-Mannheim)
and α -³²P]dCTP (NEN, Boston, MA). The filters (nylon and nitrocellulose, Pall
Filtron) were hybridized overnight at 42°C with 10⁶ cpm/ml. The washes were
20 carried out for 10 minutes at 42°C and for twice 30 minutes at 65°C in a 0.5 X
SSC buffer and 0.1 % SDS. Next, these blots were exposed to
autoradiographic films (X-Omat AR or Biomax MS, Kodak). The autoradiograms
were analysed by densitometric *scanning* (Biorad GS 670 densitometer).

25 5.2 Measurement of the transcriptional activity by run on or transcription in vitro

 The nuclei of the cells and of the tissues were prepared according to the
Nevins technique (Nevins, 1987). The total RNAs labelled with α -³²P]UTP
(3000 Ci/mmol) (NEN, Boston, MA), in quantity of equivalent radioactivity, were
hybridized on membranes on which there had been deposited 5 µg of the
30 different plasmids or 1 µg of the restriction fragments of the genes of interest.

5.3. Primer extension

The location of the site of initiation of hFATP was determined as described in Sambrook et al. (Sambrook et al., 1989). An antisense oligonucleotide, starting at +6 bases from the site of initiation of translation, was used 3'-CCC GCA TCC CTT CAC GTC CCG TCT CCC-5'. 10 µg of total RNA of human adipose tissue were precipitated with 500,000 cpm of primer labelled in 5' using T4 polynucleotide kinase (Amersham, Courtaboeuf, France with γ -³²P]ATP, denatured for 5 minutes at 95 °C and annealed for 90 minutes at 65°C in a hybridization solution. The extension was carried out at 42°C for one hour with 50 U AMV-RT (*Life Technologies, Paisley, GB*) and 100 U MMLV-RT (*Life Technologies, Paisley, GB*). A control sequence reaction was used as standard mass marker to locate the 5' end of the extension product.

6. DNA techniques

6.1. Screening of complementary DNA libraries and of genomic DNA of phages

Three libraries were used to isolate the FATP gene, including a commercial cDNA library (*Human fat cell 5'-stretch plus cDNA library* HL3016b, *oligod'1-primed, Clontech laboratories, Inc. USA*). After titration of the libraries, 10⁶ plaque forming units (PFU) served for the infection of 6 ml of the respective competent bacteria (Y1090, XL-1 and SRB) OD₆₀₀=1, maintained in Luria Bertani medium with 0.2% of maltose and 10 mM MgSO₄. The adhesion of the phages to the bacterial walls was carried out for 15 minutes at 37°C in 14-ml polypropylene tubes. The mixture was then spread with TOP agar (*NZ amine, casein hydrolysate, yeast extract* (NZY) + 0.7% agarose) on 10 culture dishes 15 cm in diameter. The lysis was carried out overnight at 37°C. Two blottings on nitrocellulose filters (NEN, Boston, MA) were carried out on each dish, taking care to note the orientation of the dishes. The filters were then treated in a denaturing, neutralizing and washing solution, and then fixed under UV or with heat. The hybridization, washing and exposure conditions were the same as those used for the RNA techniques. In each screening, a double-stranded probe was used, derived from rt-PCR or from restriction. The probe was for

example a 652 pb fragment, amplified with the primer sense GM1 (ou 382) (5'-ATG CGG GCT CCT GGA GCA GGA ACA -3') and antisense GM2 (ou 399) (3'-CTG CGT GTC AGG CAG GAT GCT TCT AGG CCC-5') covering the 5' end of mFATP cDNA. The clones isolated from a first screening were then purified
5 by two additional screenings. A final step consisted in amplifying the titre of the phage clone isolated.

6.2. Subcloning of the *hFATP* phage clones

The phage DNA was then prepared according to the Sambrook
10 technique (Sambrook et al., 1989) or amplified by PCR with a *high fidelity polymerase* (Boehringer-Mannheim), and then mapped and excised at the level of the cloning site in the phage in order to then introduce it into a *bluescript* (BS) type vector, pBS-KS. The inserts could thus be amplified, sequenced, mapped and manipulated more easily.

6.3. Sequencing and alignment of sequences

The first clones were sequenced manually with the T7 sequencing kit (Pharmacia) or on a PCR product after treating the inserts with a phosphatase and an exonuclease I in order to remove the primers and the dinucleotides
20 remaining which interfere with the sequencing reaction. The product of sequencing was then deposited on a 6 or 7% acrylamide gel, the migration lasted 3-4 h at 60 mA. The sequence was read on the autoradiogram and could be as high as 200 bp.

The sequences were then prepared on an automatic sequencer (ABI
25 377, Perkin Elmer) with a PCR sequencing reaction using fluorescent terminators (ABI Prism big dye terminator, cycle sequencing ready reaction kit, Perkin Elmer Biosystem), the reading is automatic and may be as high as 700 bp. The sequence alignments and searches were carried out in a first instance with *Genbank* by means of the *Lasergene Navigator* software (DNA Star) and
30 then in a second stage using the Internet on a national center for biotechnology information (NCBI) server. The search for homology for the cDNA fragments

was carried out using the *Blast search* programme. The *online mendelian inheritance in man (OMIM)* searches made it possible to identify the genes located on the same chromosome as hFATP.

5 6.4. Search for introns

The search for the intron/exon was carried out by PCR amplification on genomic DNA by comparison with amplifications on cDNA (phage clone, plasmid) or rt-PCR. Direct sequencing on plasmid artificial chromosome (PAC) (Genome systems Inc., St. Louis, USA) were undertaken. The junctions were
10 identified after alignment of the sequences.

7. Measurement of the transport of oleate

7.1. Preparation of the fatty acids

The labeled fatty acids (^{14}C oleate, 40-60 mCi/mmol, NENE) are
15 incubated in the presence of albumin in a ratio of between 0.5 and 2 for 45 minutes at 37°C in 1X Hank's solution.

7.2. Measurement of transport

The cells treated with the various activators are rinsed with a Hank's
20 solution and then incubated for 1 h with serum-free and glucose-free medium. The cells were again rinsed with a 1X Hank's solution with 0.2% of *bovine serum albumin* (BSA) at 37°C and then at 23°C, the transport being measured at room temperature. The incubation of the cells with labeled oleate lasts for 1 minute, the stop solution was an ice-cold Hank's solution. The cells were lysed
25 with 0.1% SDS, the proteins were assayed on 20µl, the remainder was supplemented with 4 ml of scintillation liquid and counted.

8. Chromosomal location

The FATP probe was labelled with digoxigenine-11-dUTP by nick
30 translation (Boehringer-Mannheim). The probe was purified and concentrated by precipitation in order to obtain a concentration of 50 ng/µl. The chromosomal

preparation was obtained from T lymphocytes stimulated with phytohaemagglutinin. The cells thus stimulated were synchronized after 48 h of culture by the action of methotrexate for 17 h and then the cell cycle was unblocked by the addition of thymidine. After 4h 30 min of action, the cells were
5 blocked in the metaphase by the action of colcemid. After a hypotonic shock and fixing, the preparation was deposited on a slide; to obtain good precision, the techniques of G bands obtained by the action of trypsin and after Giemsa staining (GTG banding) (Francke et al., 1978) and the FISH technique were combined. The metaphases were then selected on a DMRXA microscope
10 (Leica). They were karyotyped and stored in a software for image analysis (CHANTAL) developed by Leica. After non-isotopic in situ hybridization (Geffroy et al., 1995) with the FATP probe and detection, the metaphases were examined with the aid of a fluorescence microscope (Leica type, DMRXA) combined with a Leica image analyzing system. The software allowed
15 superposition of the metaphase in the G band and in FISH.

2. ISOLATION AND CHARACTERIZATION OF THE HUMAN *FATP* GENE

1. Results

1.1. Isolation of the complete cDNA from the human gene for *FATP*

20 1.1.1. Purification of the hFATP clones

Several human adipose tissue cDNA libraries, (including one commercial human fat cell 5' stretch plus cDNA library HL3046b, oligodT primed, Clontech library) were screened with a murine probe in order to isolate various cDNA clones of the human *FATP* gene. The first screening was carried out on the
25 commercially made human adipose tissue cDNA library (Clontech). The probe used was a 5' fragment of 654 bp (position 1-654) amplified by rt-PCR on mouse adipose tissue RNA with primers 382 (5'-ATG CGG GCT CCT GGA GCA GGA ACA-3') and 399 (3'-CTG CGT GTC AGG CAG GAT GCT TCT AGG CCC-5') or alternatively (GTG TCA GGC AGG CAG GAT GCT CTC). This
30 probe was inserted into a *bluescript* plasmid, pBS-KS, at the level of the EcoRV site and the sequence of this plasmid, mFATP399, revealed 100% homology

with the murine sequence. The screening provided 23 positive clones from 10^6 PFU, the probability of isolating a gene having a normal frequency being 1 out of 1 million. Next, a second *screening* was undertaken on another library under the same conditions for titration and spreading of the phages and for hybridization. This screening provided 6 clones which were identified as containing *FATP*. These clones were purified over three successive screenings.

The cDNA inserts, excised after digestion with *EcoRI*, provided fragments in a size interval from 300 bp to 2 kb. These clones were amplified by PCR using the lambda phage gt11 primers (λ gt11 *Forward* and λ gt 11 *Reverse*). The sequencing of these PCR products allowed us to differentiate 1) the clones whose DNA sequences were homologous to *mFATP*; they are the partial *hFATP* clones, 2) the clones whose sequence was overlapping or even identical and 3) the clones whose DNA sequence did not align with *mFATP*. The latter category comprises false-positives which proved positive in hybridization because of homology with the probe used but whose sequence c
differentiates them from *hFATP*. They may be genes already identified or *open*
reading frames (ORF) not yet identified.

Three large inserts, homologous to *mFATP* (1.6 kb clone 2.a, 2 kb, clone 3.e and 1 kb, clone 3.g starting at nucleotide +84 following the ATG codon), were subcloned to a *Bluescript* vector at the level of the *EcoRI* site and completely sequenced over the sense and antisense part. These fragments were then aligned with the mouse sequence which was, with the rat sequence *rFATP*, highly homologous, the only two sequences known from 1994 to the end of 1997. The 1.6 kb clone aligned from position 487 on mice up to position 1941 which corresponds to the TGA codon, that is to say the end of the murine coding part. This clone therefore had an additional 3' part (147 bp) which was identified by rt-PCR as the 3'*untranslated transcriptional region* (3'UTR) and of the *FATP* gene, a region which is transcribed but not translated.

The 2 kb clones appeared to be identical during total amplification of these 2 kb with the λ gt11F and λ gt11R primers by PCR. The sequencing of these clones made it possible to differentiate them: *hFATP1* and an alternative

form of splicing. The open reading frame of this clone is 1941 bp for *hFATP1*, that is to say identical to the murine coding sequence of 1941 bp. Its alternative form of splicing had an 82-base deletion compared with *hFATP1*. This deletion is situated from position 168 to 249 on the mouse sequence or the *hFATP1* sequence. This deletion could result from an alternative splicing, which was thus analyzed with the genomic structure. These two clones had 22 bases of 5'UTR and 265 bases of 3'UTR.

1.1.2. Characteristics of the clones

A search in the NCBI library revealed 84% similarity at the nucleotide level between *hFATP1* and *MMU15976* (murine *FATP* sequence identification name) and 83% between *hFATP1* and *RNU89529* (rat *FATP* sequence identification name). The murine *FATP* sequence exhibits 93% at the nucleotide level with the *rFATP* sequence. A search for sequence homology with the entire library of genes listed by NCBI was carried out with the Blast search program. This analysis revealed 57% homology at the nucleotide level with the cDNA for rat acyl coenzyme A synthetase (D85100) with an alignment over 351 bases, 58% with the murine homologue of ACS (AF33031) with an alignment over 348 bases. The *hFATP1* sequence also exhibited 94% identity over 18 bases (D88308) and 56% over 210 bases with the human homologue of ACS. The center of this region of homology is indicated in Figure 2.

At the amino acid level, the protein exhibits 89% homology with the murine protein, mFATP, and 89% with the rat protein rFATP. rFATP and mFATP have 93% homology. Yeast Fat 1P had, for its part, only 54% homology with mFATP.

The hFATP protein comprises 646 amino acids, its calculated molecular weight is 71 kDa and its isoelectric point is 8.5. We compared the amino acid sequence of *hFATP1* and from its alternative splicing form. Said alternative from encodes a protein of 57 amino acids and ends with an ambre mutation which stops translation. By observing the translation in the three phases of the complete sequence, we noticed that the sequence following this termination

site aligned farther away in another reading frame with *hFATP1*. Such a phenomenon can only be due to a splicing error which can be confirmed by analyzing the genomic structure.

Analysis of the composition of the protein by the DNA Star software shows 40% of hydrophobic amino acids, which is in agreement with the fact that FATP is a transmembrane protein. *hFATP* has a signal sequence of 30 amino acids (von Heijne, 1986). The amino acid sequence of *hFATP* shows three potential glycosylation sites (N-X-S/N-X-T), these sites are conserved in mice, rats and humans. Only one of these sites is identical to the yeast Fat 1P protein whose amino acid sequence was published in 1997 (Faergeman et al., 1997). In a very advantageous manner, a sequence of eleven amino acids is perfectly identical and conserved between *hFATP*, *mFATP*, *rFATP*, Fat 1 and *rACS*. This oligopeptide (IYTS GTTGLPK) is common to members of the family of AMP-binding proteins. On the basis of this sequence conservation, the common evolutionary line for the *FATP*, Fat 1P and *ACS* genes can be confirmed.

The structural units characterizing the secondary structure of the *FATP* protein was studied by comparing its protein analysis using the protean program of the DNA Star software (Figure 6).

Using the kyte and Doolittle algorithms, the hydrophilicity profiles of *hFATP* and *mFATP* was compared (kyte et al., 1982). An analysis of the primary amino acid sequence based on the Chou-Fasman calculations was then carried out in order to locate the α and β sheets, and the bent and helical regions (Chou et al., 1974). This study was complete with a Garnier-Robson analysis locating the same regions but with a different method (Garnier et al., 1978). These analyses demonstrated that these proteins exhibited comparable profiles.

The 3'UTR region does not contain putative polyadenylation sites or destabilizing consensus sequence (ATTTA), which suggests that the complete message is much longer than 2 kb. This hypothesis was confirmed further to a commercial Northern-blot hybridization with a human *FATP* probe. The size of

the messenger is approximately 4.4 kb.

1.1.3. Identification of the site of initiation of transcription

In order to identify the site of initiation of transcription, a 5' extension,
5 *primer extension*, with an antisense primer (reverse GM6) positioned in 5' of the
coding region was carried out. The number of bases between the primer and
the extension product was 34 bases, the primer was 27 bases long, which
locates the site of initiation 61 bases upstream of the ATG. This distance is
relatively short compared with the average distances of the sites of initiation
10 which, in addition, may be highly variable. The location was checked with a
rapid amplification of cDNA ends (RACE) technique.

1.2. Isolation of the human FATP gene and genomic characterization

1.2.1. Isolation of the human genomic DNA clone

15 In the second instance, a human genomic DNA phage library was
hybridized with a human probe and 6 genomic clones were isolated from 10^6
PFU. The genomic characterization of human *FATP* was carried out by CPR on
the genomic clones and total genomic DNA.

A PAC clone, derived from a plasmid capable of inserting fragments up
20 to 120 kb was used. This clone was obtained using two primers, the first
located from nucleotide 1267 to 1288 (GM3) and the second from nucleotide
1495 to 174 or more preferably from nucleotide 1469 to 1489 (GM4) in the
sequence. The *screening* was carried out by PCR on human genomic DNA.
The determination of the introns on this PAC was carried out by automatic
25 sequencing using a sequencing technique developed for long DNA fragments
(Fajas et al., 1997). Unlike the genomic clones previously isolated, the
PAC14957 clone did not contain the pseudogene. Indeed, the primers used
were intended to amplify the region which contained an intron, which made it
possible to avoid again isolating the pseudogene. For the remainder of the
30 characterization, this PAC14957 clone and the human total genomic DNA being
checked were used.

1.2.2. Structure of the gene

On this PAC clone, the introns situated at the 3' end of the FATP gene up to position 997 were identified. Beyond this region, towards the 5' end, no alignment could be achieved on more than 3000 bp sequenced on a PAC 14957 clone by the technique of DNA walking towards the 5' region. Furthermore, this PAC clone did not appear to hybridize with oligonucleotides having the 5' part of the cDNA. In order to determine the positions of the other introns, a PCR strategy was applied to human genomic DNA in comparison with the rt-PCRs carried out on the human adipose tissue RNA (Figure 8). In order, the size of the exons is: 167, 395, 162, 70, 92, 111, 209, 127, 138, 165, 147 and 158 bp.

1.3. Chromosomal location of *FATP*

With the aim of determining the chromosomal location of *hFATP*, an *in situ* hybridization technique was used. Twenty interpretable metaphases, following the chromosomal preparation of T lymphocytes, were selected for hybridization with the *hFATP* probe. The *in situ* hybridization with the PAC14957 probe, a genomic clone of *hFATP*, labelled with digoxigenin, was located on chromosome 19, more precisely 19p13.1.

This region is very rich and 126 genes or locus responsible for disease were located in this region. Among the genes of greatest interest, at the level of the lipid and carbohydrate metabolism, situated in the same 19p13 region, there are: the familial hypercholesterolaemic locus, the insulin receptor, the atherogenic lipoprotein phenotype, apolipoprotein C-I and the locus involved in apo C-II deficiency.

2. Discussion

The human homologue of *mFATP* was isolated by screening a human adipose tissue cDNA library. Two messengers were differentiated: *hFATP1* which encodes a protein of 646 amino acids and *hFATP2*, the truncated form of

hFATP1, which encodes a protein of 57 amino acids. The functionality of *hFATP2* is very unlikely and is derived from an alternative splicing. *hFATP1* comprises 12 exons and 11 introns.

The *hFATP1* protein is hydrophobic in nature and its protein profile is very similar to that of *mFATP*. *hFATP1* is a hydrophobic, transmembrane protein with three glycosylation sites and a potential signal sequence of 30 amino acids. Analysis of sequence homologies with other proteins made it possible to reveal 11 amino acids which are perfectly identical between *ACS* and *FATP*. These amino acids would constitute a binding site common to these two proteins. Shaffer and Lodish had already suggested that *FATP* and *ACS* could act in concert; the inventors have now shown analysis of gene regulation that *FATP* and *ACS* are regulated in a coordinated manner in tissues sensitive to insulin. *FATP* is thought to allow the entry of fatty acids into the cell and *ACS* is thought to convert them to active metabolic compounds, the acyl CoAs, for storing energy as a reserve or for the production of energy.

The location of *FATP* gene on chromosome 19, more precisely 19p13.1, is of interest since this region comprises numerous genes involved in the metabolism of fatty acids: the locus for apo C-II deficiency, the metabolism of glucose, the insulin receptor and the metabolism of cholesterol with the locus for familial hypercholesterolaemia. *FATP* is thought to form part of a syntenic group with one of these other genes and to segregate in the same manner. Thus, apo C-II deficiency causes a decrease in the LPL activity and is accompanied by accumulation of particles high in triglycerides. The insulin receptor has been very widely studied in order to search for the causes of insulin-dependent diabetes, an abnormality in this receptor prevents the action of endogenous and exogenous insulin. Familial hypercholesterolaemia due to a mutation in the receptor for the LDL particles causes the development of atherosclerosis and of cardiovascular diseases. All these manifestations have in common an accumulation of lipids in the bloodstream which may lead to insulin resistance.

Example 2

INDUCTION OF THE FATP GENE BY DIMER SELECTIVE RETINOIDS
SUGGESTS THAT PPAR-RXR HETERODIMER IS ITS MOLECULAR
5 TARGET.

Free fatty acids can be released from adipocytes by the hormone-sensitive lipase (HSL) or from tripglycerides-rich lipoproteins by lipoprotein lipase (LPL). Circulating fatty acids can then cross the plasma membrane either
10 by virtue of their lipid solubility (Higgins, 1994) or be taken up by cells in a process mediated by the fatty acid transport protein (FATP). A second protein found to be involved in the process of long-chain fatty acid uptake is the Acyl Coenzyme A synthetase (ACS). FATP acts as a transporter of fatty acids, whereas the role of ACS is rather confined in preventing the efflux of fatty acids
15 through an esterification process.

FATP and ACS mRNA levels are regulated in a tissue-specific manner
by peroxysome proliferator-activated receptors (PPARs) (Martin et al., 1997). PPARs are members of the nuclear receptor gene superfamily. Expression of LPL, FATP and ACS is known to be under the control of hypolipidemic and
20 hypoglycemic drugs, an effect mediated by PPARs. Three retinoic acid receptors, termed RAR α , - β and - γ , and three retinoid X receptor, designated RXR α , - β and - γ , are classically thought to transduce the effects of retinoic acid (RA) on gene expression. Both 9-*cis* RA (9c-RA) and *all trans*-RA (at-RA) can directly bind and activate RARs, whereas RXR doesn't bind at-RA, but binds
25 9c-RA. RXR-PPAR heterodimers respond to both RXR and PPAR ligands (Kliwer et al., 1992).

RXR agonists function as insulin sensitizers and have beneficial effects on hypertiglyceridemia, hyperglycemia and hyperinsulinemia in mouse models of NIDDM and obesity (Mukherjee et al., 1997). This effect is not secondary to a
30 variation in the weight of the animals. Furthermore, some of these rexinoids allow to determine which heterodimer is specifically involved in gene regulation

and hence these ligands provide a new tool for a better understanding of RXR action and the development of new pharmacological compounds.

Because thiazolidinediones, which are PPAR γ specific ligands, are thought to exert part of their antidiabetic effect through an alteration of the fatty acid partitioning and activation of PPAR γ -RXR, the inventors proposed that RXR ligands could have similar effects and be potential activators of FATP, LPL, and ACS expression. LPL, FATP, and ACS are involved in fatty acid partitioning and the levels of circulating free fatty acids depend on the expression of these genes. PPAR γ activators improve glucose homeostasis and this effect may be due to the regulation of LPL (Schoonjans et al., 1996), FATP and ACS target genes. In order to confirm the implication of PPAR-RXR heterodimer in the improvement of glucose homeostasis, the regulation of LPL, FATP, and ACS genes by retinoic acid was studied. This allowed to determine which dimer was involved in this process and provided a better understanding of the regulation of lipid uptake by nuclear receptors. The PPAR-RXR heterodimer as the molecular target mediating these effects on fatty acid partitioning leading to an improvement of insulin sensitivity.

1. Material and methods

Abbreviations

RA, retinoic acid; at-RA, all-*trans* RA; 9c-RA, 9-cis RA; RAR, Retinoic acid receptor; RXR, retinoid X receptor; RXR-RE, RXR response element; TTNPB, ethyl-p-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphtyl-1-propenyl)]benzoic acid; PPAR, peroxisome proliferator-activated receptor; DMSO, dimethyl sulfoxide.

Materials

at-RA was purchased from Sigma (St. Louis, Mo.) 9c-RA, TTNPB (Mangelsdorf et al., 1990) was obtained from ligand pharmaceuticals.

Cell culture and treatments

FAO cells are a well-differentiated subclone derived from the rat hepatoma H4 IIEC3 line. They were cultured at 37°C in a humidified atmosphere of 5% CO₂, 95% air in Ham F-12 medium (Gibco-BRL) containing 10% fetal calf serum, penicillin (200 IU/ml) and streptomycin (50 mg/ml) according to previously published procedures (Meunier-Dumort et al., 1996). Culture medium was changed every 48 hr. Experiments were performed on subconfluent cells maintained in culture under serum free conditions. at-RA and 9c-RA were dissolved in dimethyl sulfoxide (DMSO) while oleic acid was complexed with bovine serum albumine before addition to the cells (BSA). The human hepatoma cell line HepG2 was obtained from ECACC (Porton Down, Salisbury, United Kingdom) and the mouse preadipocyte cell line 3T3-L1 from (ATCC). These cells were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM), supplemented with 10% fetal calf serum (LPDS-DCC), L-glutamine and antibiotics, unless stated otherwise. 3T3-L1 cells were differentiated initially by a 2 day-treatment with dexamethasone (0.1µM), isobutyl methyl xanthine (0.25mM) and insulin (0.4 µM). Subsequently, the cells were then maintained for an additional 8 days with insulin until complete differentiation. Experiments were performed on subconfluent cells maintained in culture under serum free conditions. at-RA and 9c-RA were dissolved in dimethyl sulfoxide (DMSO) while oleic acid was complexed with bovine serum albumine (BSA) before addition to the cells.

RNA analysis

RNA preparation, northern blot hybridizations, and quantification of total cellular RNA were performed as described previously (Auwerx et al., 1988). A mouse FATP cDNA probe was obtained after cloning an RT-PCR fragment from mouse adipose tissue RNA (primers ATG CGG GCT CCT GGA GCA GGA CAG CC and CTG CGT GTC AGG CAG GAT GCT CTC AGG CCC) into

pBluescript-KS. The insert was sequenced and found to be identical to the reported mouse FATP sequence. The rat ACS probe corresponds to the EcoRV restriction fragment of the rat ACS cDNA and an EcoRI fragment of the hLPL26 clone was used to measure LPLmRNA. β -actin was used as control probe.

Isolation of nuclei and transcriptional rate assay

Nuclei were prepared from FAO and 3T3-L1 differentiated cells and treated either with 9c-RA or vehicle. Transcription run-on assays were performed as described by Nevins (Nevins, 1987). Equivalent counts of nuclear RNA labeled with [α - 32 P]UTP (3000 Ci/mmol) were hybridized for 36 hr at 65°C to 5 μ g of FATP, ACS, GAPDH and vector DNA (pBluescript) immobilized on Hybond-C Extra filters (Amersham). After hybridization, filters were washed at room temperature for 10 min in 0.5xSSC and 0.1% SDS and twice at 65°C for 30 min and subsequently exposed to X-ray film (BIOMAX-MS, Kodak). Quantitative analysis was performed by scanning densitometry (BioRad GS670 densitometer).

Preparation of albumin-bound fatty acids and Fatty acid uptake assay

Radiolabeled 14 [C] oleate fatty acid was added to water at 40°C. Albumin (BSA; fraction V, fatty acid free, Sigma, St Louis, Mo) was then added from a concentrated stock (20 g/100 ml) to give a final molar ratio of 1/1 by gentle mixing. 2 X Hank's solution was added to obtain a 1 X final solution. Incubation was carried out at 37°C for 45 minutes.

The measurement of uptake of 14 [C]-labeled oleate (about 50 mCi/mmol, NEN, Boston, MA) was carried out in 24 or 6 well plates with 10^6 cells / ml of medium. Before treatment, the cells were washed with 1X Hank's solution. BRL 49653 (100 - 250 nM) and fenofibric acid (100 - 250 μ M) and the other retinoids (dose range from 10^{-9} M to 10^{-6} M) were added in fresh DMEM medium containing 10% FCS. After 48 h of treatment, cells were washed with Hank's solution and incubated for one additional hour in serum-free, glucose-

free medium. Cells were then washed once at 37°C and twice at 23 °C with 1X Hanks solution containing BSA. Hank's solution without BSA was then added before the assay. A volume corresponding to 1µCi of ¹⁴[C]-oleate albumin-bound solution was added in each well and cells were incubated for 1 min at room temperature. Incubation was stopped after 1 min with 3 washes of ice cold 1X Hank's solution without BSA. A complementary experiment has been performed to verify whether a specific cell surface binding of ¹⁴[C]-oleate could interfere with the assay. For this second assay, the cells were washed under more stringent conditions in 1X Hank's solution containing 0.5% BSA. Cells were then lysed in 400 µl of 0.1% SDS solution. The lysate was counted for 5 min with 4 ml of scintillation solution. Assay was performed on triplicate points.

Results

15 1.9c-RA induces FATP and ACS mRNA levels in the hepatoma cells FAO and HepG2

In order to determine whether FATP and ACS genes were regulated at transcriptional level by retinoic acid, a dose-response experiment was performed. FAO cells were cultured for 6 hr with increasing doses of 9c-RA (10⁻⁸ to 10⁻⁴ M) whereas control cells were incubated with vehicle alone (0,1% v/v DMSO). Incubation with 9c-RA resulted in a dose-dependent increase of both FATP and ACS mRNA levels in this hepatic cell line (figure 10A). A maximal increase for FATP (>9-fold) and ACS (14-fold) was observed with a dose of 10⁻⁶M 9c RA. FATP and ACS m RNA are also upregulated by at-RA but to a lesser extent than 9c-RA (data not shown).

Induction of FATP and ACS mRNA levels in the human hepatoma cell line HepG2 was analyzed. 9c-RA induced FATP and ACS mRNA after 24 hr (dose 10⁻⁸ M) (figure 10B). However, no effect of at-RA could be detected (data not shown).

2. 9c-RA induces FATP and ACS mRNA levels in differentiated Caco2 and 3T3-L1 cells.

Since adipose tissue is an important insulin-sensitive tissue, it was
5 interesting to know whether retinoids also regulate FATP and ACS gene
expression in mouse adipose cell line, 3T3-L1. 3T3-L1 cells were therefore
completely differentiated and then treated with 9c-RA (10^{-9} to 10^{-6} M). 9c-RA
induces FATP and ACS mRNA expression in a dose-dependent manner.
Maximum increase was observed at a concentration of 10^{-6} M 9c-RA (figure
10 11A).

The expression of FATP and ACS induction was next compared between
Fao hepatoma cells and the colon adenocarcinoma cells Caco2. Both FATP
and ACS gene expressions are increased after 6 hr of treatment with 9c-RA at
 10^{-6} M. However, higher levels of induction are nevertheless observed in FAO
15 cells (figure 11B).

3. The induction of FATP and ACS gene expression by 9c-RA is at the transcriptional level.

Nuclear run-on analysis was next carried out to determine whether the
20 induction of this expression by 9c-RA was a direct consequence of enhanced
gene transcription. FAO cells were therefore treated for 2 hr with 9c-RA (10^{-6} M)
after 18 hr of culture in serum-deprived medium, whereas 3T3-L1 cells were
treated for 24 hr with 9c-RA (10^{-6} M) in normal medium without serum
deprivation. FAO metabolism is greatly affected with lipids, serum deprived
25 conditions allow to observe a strong effect which couldn't be detected in normal
conditions. Controls were treated with vehicle only. Transcription rates for the
FATP and ACS genes were induced 2-fold and 2.8-fold respectively in FAO
cells and 4- and 3-fold respectively in differentiated 3T3-L1 cells. In the retinoic
acid treated cells, transcription of the GAPDH gene was not affected in neither
30 of the two cell lines.

4. Rexinoids, but not RAR agonists, induce FATP, ACS and LPL gene expression in differentiated 3T3-L1 cells.

5 To determine whether the effect of retinoids on the regulation of FATP and ACS gene expression was mediated by RAR or RXR, 3T3-L1 differentiated cells were treated with 9c-RA, a panagonist of both RAR or RXR, or at-RA and TTNPB which are specific agonists of RAR. No activation of LPL, FATP and ACS gene expression was detectable after 24 hr of treatment with at-RA or
10 TTNPB. In contrast, a strong dose-dependent induction of both LPL, FATP and ACS was observed 24 hr after treatment with 9c-RA, a RXR agonist. Maximum induction occurred at 10^{-6} M 9c-RA (4-fold and 3-fold for LPL, FATP and ACS respectively). Interestingly, no effect of non of the retinoids was observed on non-differentiated 3T3-L1 preadipocyte cells (figures 13, 14 and 15).

15 RXR agonists, but not RAR agonists, induce oleate uptake in differentiated 3T3-L1 cells.

To establish whether the induction of the FATP-1 and ACS was accompanied
20 by a concomitant increase in fatty acid uptake into the cells, we measured 14 [C] oleate uptake in differentiated 3T3-L1 cells exposed to increasing doses of prototypic retinoid, i.e., TTNPB, an RAR agonist (dose range 10^{-9} M to 10^{-6} M). No effect on 14 [C] oleate uptake was observed after TTNPB confirming that this effect was specific for the rexinoids (figure 16).

25 Discussion

These results demonstrate that RXR ligands control both FATP and ACS gene expression in several cell types. In the liver, FATP and ACS have been
30 shown to be strongly upregulated by PPAR α activators (Schoonjans et al., 1995; Martin et al., 1997). These distinct transcriptional effects are mediated by

PPAR α interacting with responsive elements in the promoter regions of these genes. As PPAR α effects are mediated through activation of the PPAR α -RXR heterodimer, it was not surprising to observe a strong induction of FATP and ACS expression by specific RXR activators. Expression of FATP and ACS are co-induced by 9c-RA in the liver and in intestine. Free fatty acids generated by LPL are avidly taken up by these tissues and are converted in metabolic active acyl-CoA derivatives to sustain a high level of β -oxydation. The co-induction of FATP and ACS by 9c-RA, which resembles the induction of these genes by fibrates, indicates that 9c-RA and fibrates have a similar metabolic effect on rat and human hepatoma cell line (Kliwer et al.). These data demonstrate that the RA pathway is implicated in the regulation of lipid metabolism and underline the powerful action of specific RXR agonists on the uptake and metabolism of long-chain fatty acids in the liver.

In adipose tissue, a second insulin-sensitive tissue, FATP and ACS are also co-regulated by 9c-RA in a dose-dependent manner. PPAR γ is the predominant in this tissue and PPAR γ activators have been shown to induce both FATP and ACS expression. Hence, the induction of FATP and ACS expression by RXR agonists in the context of PPAR γ -RXR heterodimer suggests that RXR is also here an active heterodimer partner.

The stimulatory effects of 9c-RA on FATP and ACS gene expression in both tissue implicate the RA pathway as an important regulation pathway in liver and adipose tissue where retinoids have a significant physiological role in fatty acid metabolism. Since FATP and ACS genes are implicated in fatty acid partitioning, it can be concluded that the distribution of fatty acids is affected in response to 9c-RA. This effect of rexinoids on fatty acid partitioning is different from the effects observed with PPAR activators and depends on the relative expression of the different RXRs and PPARx (Braissant et al., 1994; Lemberger et al. 1996; Auboeuf et al., 1997). RXR is present in both liver and adipose tissue and this pattern of expression determines the tissue specific effects of RA activation. In contrast, activity of PPAR α agonists is mostly limited to the

liver, the prime site of expression of PPAR α . Furthermore, the fact that the co-regulation of LPL, FATP and ACS genes by 9c-RA, TTNPB and at-RA depend on the differentiated state of 3T3-L1 cells points to the importance of PPAR γ in this process. In preadipocyte cells PPAR γ is expressed at low levels and its expression increases upon adipocyte differentiation. The absence of a retinoic acid response in differentiated 3T3-L1 cells expressing almost no PPAR γ suggest that PPAR γ is an obligatory partner of RXR in the retinoid-dependent regulation of LPL, FATP and ACS genes in adipose tissue. Hence, due to its more general expression, it is expected that the effects of rexinoids are more generalized than the effects of the more tissue-restricted PPAR α (liver) and PPAR γ (adipose tissue) agonists. In fact, rexinoids effects on gene expression should resemble the effects of a combined PPAR α and γ agonist. This effect is direct on the FATP gene and suggests the presence of a response element like it has been demonstrated in ACS and LPL gene (Schoonjans et al., 1996) but also and recently in FABP gene (Poirier et al., 1997).

It seems that rexinoids, the new term used to specify RXR selective ligands collaborate with PPAR ligands to control the expression of FATP and ACS genes involved in fatty acid metabolism. The beneficial effect of PPAR γ activators on glucose homeostasis has been previously shown by us to be in part due to a redistribution of fatty acids towards adipose tissue with a relative depletion of fatty acids in the muscle (Martin et al., 1998). As known since Randle's work in the 60 (Randle et al., 1963; Randle et al., 1964), depletion of muscle fatty acid content will result in an improvement of glucose homeostasis. Hence, it is tempting to speculate that the improvements of glucose homeostasis observed with rexinoids are similarly linked to an altered partitioning of fatty acids. In view of the distinct tissue distribution of RXR expression, induction of FATP and ACS by RXR ligands might not have the same tissue-specificity as that of PPAR α and PPAR γ activators and could contribute to a different tissue partitioning of fatty acids. These agents provide hence an alternative way to regulate the expression of genes implicated in fatty

acid distribution among the different tissues.

This study demonstrates that RXR ligands regulate the expression of LPL, FATP and ACS genes in several cell types. The retinoic acid pathway is an important signalling pathway for the regulation of genes which are implicated in fatty acid supply. Our results suggest that the PPAR-RXR complex is the molecular target by which rexinoids regulate FATP and ACS gene expression. Furthermore, they define FATP and ACS as new target genes in the RXR-dependent signalling pathway. The observation that both rexinoids and peroxisome proliferators stimulate the transcription of these genes implicated in lipid metabolism, suggest that rexinoids may act as an hypolipidemic and hypoglycemic agent through activation of PPAR-RXR complex and are consistent with the hypothesis that the heterodimer PPAR-RXR heterodimer is the molecular target for the improvement of insulin sensitivity.

TTNPB, which is an activator of RAR in the context of RXR-RAR heterodimer, has no effect on FATP-1 and ACS. The combined results of the studies using these synthetic retinoids implicates the PPAR-RXR heterodimer as the

molecular target of the regulation of FATP-1 and ACS by retinoic acids. Since PPAR and RXR are both active components of this heterodimer and since we have previously demonstrated that the FATP-1 and ACS genes are both regulated by PPAR activator. (Martin, G. et al (1997) J. Biol. Chem. 272:28210-7), it appears that rexinoids could cooperate synergistically with PPAR ligands in the control of the expression of these two genes involved in fatty acid metabolism. Furthermore, the fact that the coregulation of FATP-1 and ACS genes by rexinoids depends on the differentiated state of 3T3-L1 cells points to the importance of PPAR γ in this process. In preadipocyte cells PPAR γ is expressed at low levels and its expression increases upon adipocyte differentiation. The absence of a rexinoid response in undifferentiated 2T3-L1 cells, expressing almost no PPAR γ , hence also suggest that PPAR γ is an obligatory partner of RXR in the retinoid-dependent regulation of FATP-1 and ACS genes in adipose tissue.

Example 3

Expression of FATP in human tissues

5 In order to determine whether the expression of FATP was ubiquitous or limited to some tissues, hybridization experiments were performed with a human probe radioactively labeled on a commercial Northern Blot (Clontech) containing mRNA of 8 human tissues. These tissues are heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas tissues (figure 14). The expression is
10 very high in the skeletal muscle, high in heart and pancreas, medium in brain, weak in placenta, liver, and very weak in kidneys. The human mRNA corresponding to FATP has a size of 4.4 kb. The human β -actin probe has been used for the normalization and the top signal represents the ubiquitously expressed isoform, whereas skeletal and cardiac muscle express a specific β -
15 actin isoform.

The commercial Northern Blot did not contain adipose tissue probably due to difficulties in performing its extraction. Therefore, a supplementary Northern Blot analysis was performed on various human cell lines and adipose tissue.
20 The expression of FATP was analyzed. FATP was very highly expressed in adipose tissue. To a weaker extent, FATP was expressed in hepatocyte and monocytic cell lines. The expression was detectable in chorionic carcinoma cells (Jeg3) and human colon cells (Caco2).

25 The high level of FATP mRNA in the skeletal muscle has been checked on several skeletal muscular tissues and the level of expression of the FATP mRNA in this tissue has been compared with the level in sub-cutaneous or visceral adipose tissues, liver and colon tissues (figure 17).

30 Interestingly, the expression level in the human muscle (lines 1, 2, and 3) has been confirmed at a level which is close to the level observed in adipose tissue,

which was not the case when the same experiments were performed on rodents. The expression of FATP is very high in the adipose tissue. In normal patients, the adipose tissue is sub-cutaneous. In patients suffering from obesity, the adipose tissue is visceral and sub-cutaneous. In these patients, the expression of FATP is higher at the level of the sub-cutaneous tissue.

FATP is thus expressed ubiquitously in human tissues, with a predominant expression in adipose tissue, muscle and heart.

As a transport protein, FATP would permit transportation of fatty acids in the adipose tissue in nutrition periods and would be involved in efflux of said fatty acids in fast periods. Furthermore based on the observation that FATP is highly expressed in skeletal muscle, it is concluded that FATP is very important as energy source for a tissue which is known to use mainly glucose in normal conditions. This result emphasizes a competition between glucose and long-chain fatty acids for the muscular activity.

The third tissue which expressed a high level of FATP is heart. This expression is not influenced by nutrition. In brain tissue the level of FATP is significant. In addition to its activity as energy source, FATP could participate in the incorporation of lipids for synthesis of axial membranes and myelin. In the pancreas, FATP could be the specific transport protein of long-chain fatty acids messengers essential for the regulation of the production of insulin by the pancreas.

It is especially noted that the level of FATP is especially high in tissues which are sensitive to insulin such as adipose tissues and muscle.

Western blot analysis of FATP.

Cells and tissues were homogenized in a lysis buffer of PBS containing 1% Triton X-100 (Sigma, St Louis, MO). Tissues were homogenized with a polytron

in extraction buffer containing PBS and 1% NP-40, 0,5 % sodium deoxycholate, 0,1% SDS (Sigma, St Louis, MO). In the presence of a fresh cocktail of protease inhibitors (ICN, France) (100 µg/ml AEBSF, 5 mg/ml EDTA, 1µg/ml leupeptin, 1 µg/ml pepstatin). Protein extracts were obtained by centrifugation
5 of the lysate at 4°C and then concentration was measured with the Biorad DC Protein colorimetric assay system (Biorad, France).

Protein (100 µg) were separated on a 10% polyacrylamide gel according to Laemmli [Laemmli, 1970 #1541], transferred to nitrocellulose membrane as described by Towbin [Towbin, 1979#1542] (Amersham, France), and blocked
10 overnight in blocking buffer (20 mM Tris, 100 mM NaCl, 1% Tween-20, 10% skm milk). Filters were first incubated 4 hours at 21°C with rabbit IgG anti-mFATP (10 mg/ml) developed against a FATP peptide, corresponding to amino acids from human FATP-1, and next for 1 hour at 21°C with a goat anti-rabbit IgG (whole molecule) peroxidase conjugate (Pasteur Diagnostic Sanofi,
15 France) diluted at 1/5000. The complex was visualized by chemiluminescence using with 4-chloro-1-naphtol as reagent according to the manufacturer's protocol (ECL, Amersham, France).

Northern blot analysis of different human cell lines, such as NCL-H295, THP-1, Caco2, chorionic cells and hepatoma cells shows that hFATP-1 can be
20 detected in all these cells but to a relatively lower extent than in human adipose tissue (figure 18). hFATP1 mRNA is relatively well expressed in Caco2 cells and in cells of hepatic origin.

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CLAIMS

1. Nucleotide sequence which comprises a sequence involved in the expression of the human Fatty Acid Transport Protein (hFATP) comprising the aminoacid sequence of Figure 2 or Figure 5.

2. Nucleotide sequence according to claim 1, which comprises a sequence encoding the human FATP, corresponding to or comprising the nucleotide sequence of Figure 1 or Figure 3.

3. Nucleotide sequence according to Claim 2, which is the nucleotide sequence of Figure 1 or Figure 3.

4. Nucleotide sequence according to Claim 2, which comprises an ORF sequence located between nucleotide 23 (ATG) and nucleotide 1963 (TGA) of Figure 1 or Figure 3.

5. Nucleotide sequence hybridizing in high stringency conditions, with a probe comprising 50 to 300 nucleotides including at least 6, preferably at least 9 nucleotides from the following sequence :

CGGGGAGACGGGACGTGAAGGG.

6. Nucleotide sequence according to anyone of claims 1 to 5 which is a genomic DNA.

7. Nucleotide sequence according to anyone of Claims 1 to 6, which is the gene coding for the hFATP.

8. Nucleotide sequence according to anyone of Claims 1 to 3 or 6 to 7, which comprises a transcription initiation site 61 bp upstream from the ATG codon.

9. Nucleotide sequence according to anyone of Claims 6 to 8, which contains 12 exons and 11 introns having the following structure:

794																			
167		562		724		886		997		1206		1333		1471		1636		1783	
1	2	3	4	5	6	7	8	9	10	11	12								
ATG												TGA							

5 10. Nucleotide sequence according to claim 9, which comprises the nucleotide sequence of Figure 4.

11. Nucleotide sequence according to anyone of claims 1 to 10, which comprises sequences involved in the regulation of the expression of the gene coding for the human FATP.

10 12. Nucleotide sequence according to anyone of Claims 1 to 11, which sequence either specifically hybridizes with one or both of the primers having one of the following sequences or is the amplification product obtained with the following sequences, or hybridizes in high stringency conditions with said amplification product :

15 AAGGTCAATGAGGACACAATGG,
CGAGTAGGTAGTGATCGTGCAAG.

13. Nucleotide sequence according to anyone of claims 1 to 12, which is a genomic sequence coding for FATP and which hybridizes in high stringency conditions with a probe containing around 200 bp and being preferably derived from the sequence of exon 2, said genomic nucleotide sequence comprising a sequence involved in the regulation of the gene coding for the FATP.

14. Nucleotide sequence which is a mRNA as obtained by transcription of a genomic nucleotide sequence coding for the hFATP according to anyone of Claims 6 to 13.

25 15. Nucleotide sequence according to anyone of Claims 1 to 13 which is the RNA sequence transcribed from the nucleotide sequence of figure 1 or figure 3 having 2 kb, or an RNA sequence including a nucleotide sequence of 4,4 kb hybridizing in high stringency conditions with the sequence of figure 1 or

figure 3.

16. cDNA as obtained by reverse transcription of the mRNA according to Claim 14 or 15.

17. Polynucleotide which is selected among the following sequences:

5 - AAGGTCAATGAGGACACAATGG

- CGAGTAGGTAGTGATCGTG CAG

- a sequence comprising or corresponding to sequences involved in the regulation of the expression of the gene encoding the hFATP,

10 - any fragment derived from the nucleotide sequences disclosed in figures 1, 3 or 4, for instance by deletion mutation or insertion provided the essential biological properties of the native sequences are maintained.

18. Vector comprising, inserted in a site not essential for its replication, a nucleotide sequence according to anyone of claims 1 to 16.

15 19. Vector according to Claim 18, which further comprises a reporter sequence such as the CAT gene or the luciferase gene.

20. Vector according to Claim 19, wherein the expression of the reporter gene is under the control of an heterologous regulator region.

20 21. Recombinant cell which comprises a nucleotide sequence according to anyone of claims 1 to 16 or a vector according to anyone of Claims 18 to 20.

22. Recombinant cell according to Claim 21 which is a eucaryotic cell.

23. Human Fatty Acid Transport Protein (hFATP) comprising the aminoacid sequence of figure 2 or figure 5.

25 24. Human FATP according to Claim 23, which has an isoelectric point of 8.5 and which replies to the aminoacid sequence of Figure 2 or Figure 5.

25. Human FATP which is encoded by a nucleotide sequence according to anyone of Claims 1 to 16.

26. Human FATP which is devoid from its signal peptide.

27. Human FATP according to anyone of claims 23 to 26, which is

glycosylated.

28. Antibodies specifically directed against hFATP according to anyone of Claims 23 to 27.

29. Composition for the therapeutic modulation of the intracellular level of long-chain fatty acids, which comprises a component capable of regulating the expression of the gene encoding hFATP.

30. Composition for the therapeutic modulation of the blood level of long-chain fatty acids, which comprises a component capable of regulating the expression of the gene encoding hFATP.

31. Composition for the treatment of a pathological state associated with a deficient regulation of the intracellular level of long-chain fatty acids, which comprises a component capable of regulating the expression of the gene encoding hFATP.

32. Composition according to claim 30 or 31, which comprises an inhibitor of the expression of the hFATP gene.

33. Composition according to claim 30 or 31, which comprises a compound capable of enhancing the expression of the hFATP gene.

34. Composition according to claim 33 for the treatment of cardiomyopathies or diabetes.

35. Composition according to claim 32 for the treatment of obesity.

36. Method for the screening of the effect of a determined compound, on the expression of the hFATP protein in determined cells, which method comprises the step of detecting the transcription of the mRNA in the cells or cell extracts, after contacting the cells with said determined compound, in conditions enabling the interaction between said compound and said cells.

37. Method according to claim 36, for the screening of the effect of a determined compound on the regulation of the expression of the hFATP protein in cells, which comprises:

a) measuring the level of transcription of the mRNA in cells or cell

extracts, wherein the cells have previously been contacted with the determined compound, in conditions enabling the interaction of said cells and said determined compound;

5 b) measuring the level of transcription of the mRNA in the same cells species as in step a) or on extracts of these cells, wherein these cells have not been previously contacted with the assayed compound;

c) comparing the level of transcription obtained in steps a) and b).

38. Method according to claim 36 or 37, wherein the cells used are selected among the group of liver cells, heart cells, adipose tissue cells and
10 skeletal muscle cells.

FIG. 1A

1
CGGGGAGACGGGACGTGAAGGGATGCGGGCTCCGGGTGCG hFATP1 -UTR
ATGCGGGCTCCGGGTGCG hFATP' -UTR

1
41
GGCGCGGCCTCGGTGGTCTCGCTGGCGCTGTTGTGGCTGC hFATP1 -UTR
GGCGCGGCCTCGGTGGTCTCGCTGGCGCTGTTGTGGCTGC hFATP' -UTR

41
81
TGGGGCTGCCGTGGACCTGGAGCGCGGCAGCGGCGCTCGG hFATP1 +UTR
TGGGGCTGCCGTGGACCTGGAGCGCGGCAGCGGCGCTCGG hFATP' +UTR

81
121
CGTGTACGTGGGCAGCGCGGCTGGCGCTTCCTGCGCATC hFATP1 +UTR
CGTGTACGTGGGCAGCGCGGCTGGCGCTTCCTGCGCATC hFATP' +UTR

121
161
GTCTGCAAGACCGCGAGGCGAGACCTCTTCGGTCTCTCTG hFATP1 +UTR
GTCTGCAAGACCGCGAGGCGAGACCTCTT----- hFATP' +UTR

161
201
TGCTGATCCGCGTGCGCCTGGAGCTGCGGCGGCACCAGCG hFATP1 +UTR
----- hFATP' +UTR

189

241
TGCCGGCCACACCATCCCGCGCATCTTTCAGGCGGTAGTG hFATP1 +UTR
-----GCGGTAGTG hFATP' +UTR

189
281
CAGCGACAGCCCGAGCGCCTGGCGCTGGTGGATGCCGGGA hFATP1 -UTR
CAGCGACAGCCCGAGCGCCTGGCGCTGGTGGATGCCGGGA hFATP' -UTR

199
321
CCGGCGAGTGCTGGACCTTTGCGCAGCTGGACGCCTACTC hFATP1 +UTR
CCGGCGAGTGCTGGACCTTTGCGCAGCTGGACGCCTACTC hFATP' -UTR

239
361
CAATGCCGTAGCCAACCTCTTCCGCCAGCTGGGCTTCGCG hFATP1 +UTR
CAATGCCGTAGCCAACCTCTTCCGCCAGCTGGGCTTCGCG hFATP' -UTR

279
401
CCGGGCGACGTGGTGGCCATCTTCCTGGAGGGCCGGCCGG hFATP1 -UTR
CCGGGCGACGTGGTGGCCATCTTCCTGGAGGGCCGGCCGG hFATP' -UTR

319
441
AGTTCCTGGGGCTGTGGCTGGGCCTGGCCAGGCGGGCAT hFATP1 -UTR
AGTTCCTGGGGCTGTGGCTGGGCCTGGCCAGGCGGGCAT hFATP' -UTR

359

FIG. 1B

481
GGAGGCCGCGCTGCTCAACGTGAACCTGCGGCGCGAGCCC hFATP1 +UTR
GGAGGCCGCGCTGCTCAACGTGAACCTGCGGCGCGAGCCC hFATP' +UTR
399

521
CTGGCCTTCTGCCTGGGCACCTCGGGCGCTAAGGCCCTGA hFATP1 +UTR
CTGGCCTTCTGCCTGGGCACCTCGGGCGCTAAGGCCCTGA hFATP' +UTR
439

561
TCTTTGGAGGAGAAATGGTGGCGGCGGTGGCCGAAGTGAG hFATP1 +UTR
TCTTTGGAGGAGAAATGGTGGCGGCGGTGGCCGAAGTGAG hFATP' +UTR
479

601
CGGGCATCTGGGGAAAAGTTTGATCAAGTTCTGCTCTGGA hFATP1 +UTR

641
GACTTGGGGCCCGAGGGCATCTTGCCGGACACCCACCTCC hFATP1 +UTR

681
TGGACCCGCTGCTGAAGGAGGCCTCTACTGCCCCCTTGGC hFATP1 +UTR

721
ACAGATCCCCAGCAAGGGCATGGACGATCGTCTTTTCTAC hFATP1 +UTR

761
ATCTACACGTGCGGGGACCACCGGGCTGCCCAAGGCTGCCA hFATP1 +UTR

801
TTGTGCTGCACAGCAGGTACTACCGCATGGCAGCCTTCGG hFATP1 +UTR

841
CCACCACGCCTACCGCATGCAGGCGGCTGACGTGCTCTAT hFATP1 +UTR

881
GACTGCCTGCCCCCTGTACCACTCGGCAGGAAACATCATCG hFATP1 +UTR

921
GCGTGGGGCAGTGTCTCATCTATGGGCTGACAGTCGTCCT hFATP1 +UTR

961
CCGCAAGAAATTCTCGGCCAGCCGCTTCTGGGACGACTGT hFATP1 -UTR

1001
ATCAAGTACAACCTGCACGCTGCTTCAGTACATCGGGAGA hFATP1 -UTR

1041
TCTGCCGCTACCTGCTGAAGCAGCCGCTGCGCGAGGCCGA hFATP1 -UTR

FIG. 1C

1081

GAGGCGACACCGCGTGC GCCTGGCGGTGGGGAACGGGCTG hFATP1 -UTR

1121

CGTCCTGCCATCTGGGAGGAGTTCACGGAGCGCTTCGGCG hFATP1 -UTR

1161

TACGCCAAATCGGGGAGTTCTACGGCGCCACCGAGTGCAA hFATP1 -UTR

1201

CTGCAGCATTGCCAACATGGACGGCAAGGTCGGCTCCTGT hFATP1 +UTR

1241

GGTTTCAACAGCCGCATCCTGCCCCACGTGTACCCCATCC hFATP1 +UTR

1281

GGCTGGTGAAGGTCAATGAGGACACAATGGAGCTGCTGCG hFATP1 -UTR

1321

GGATGCCCAGGGCCTCTGCATCCCCTGCCAGGCCGGGGAG hFATP1 +UTR

1361

CCTGGCCTCCTTGTGGGTCAGATCAACCAACAGGACCCGC hFATP1 +UTR

1401

TGCGCCGCTTCGATGGCTATGTCAGCGAGAGCGCCACCAG hFATP1 +UTR

1441

CAAGAAGATCGCCACAGCGTCTTCAGCAAGGGCGACAGC hFATP1 +UTR

1481

GCCTACCTCTCAGGTGACGTGCTAGTGATGGATGAGCTGG hFATP1 +UTR

1521

GCTACATGTACTTCCGGGACCGTAGCGGGGACACCTTCCG hFATP1 +UTR

1561

CTGGCGAGGGGAGAACGTCTCCAACACCGAGGTGGAGGGC hFATP1 -UTR

1601

GTGCTGAGCCGCCTGCTGGGCCAGACAGACGTGGCCGTCT hFATP1 -UTR

1641

ATGGGGTGGCTGTTCCAGGAGTGGAGGGTAAGGCAGGGAT hFATP1 -UTR

1681

GGCGGGCGTCGCAGACCCCCACAGCCTGCTGGACCCCAAC hFATP1 -UTR

1721

GCCATATACCAGGAGCTGCAGAAGGTGCTGGCACCCCTATG hFATP1 -UTR

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FIG. 1D

1761
CCCCGGCCCATCTTCCTGCGCCTCCTGCCCCAGGTGGACAC hFATP1 +UTR

1801
CACAGGCACCTTCAAGATCCAGAAGACGAGGCTGCAGCGA hFATP1 +UTR

1841
GAGGGCTTTGACCCACGCCAGACCTCAGACCGGCTCTTCT hFATP1 +UTR

1881
TCCTGGACCTGAAGCAGGGCCACTACCTGCCCTTAAATGA hFATP1 +UTR

1921
GGCAGTCTACACTCGCATCTGCTCGGGCGCCTTCGCCCTC hFATP1 +UTR

1961
TGAAGCTGTTCTCTACTGGCCACAACTCTGGGCGTGGT hFATP1 +UTR

2001
GGGAGAGGCCAGCTTGAGCCAGACAGCGCTGCCCAGGGGT hFATP1 +UTR

2041
GGCCGCCTAGTACACACCCACCTGGCCGAGCTGTACCTGG hFATP1 +UTR

2081
~~CACGGCCCATCCTGGACTGAGAACTGGAACCTCAGAGGA hFATP1 +UTR~~

2121
ACCCGTGCCTCTCTGCTGCCTTGGTGCCCTGTGTCTGCC hFATP1 +UTR

2161
TCCTCTCCCTGCTTTTCAGCCTNTGTCTCCTTCCATCCNT hFATP1 +UTR

2201
GTCCCTGTNTGGCCTTAACCCG hFATP1 +UTR

1 M R A P G A G **A** A S V **V** S L A L L W **L** L hFATP1
ATG CCG GGT CCG GGT CCG GCG GCG TGG GTG GTC TGG CTG CCG CCG TGG TGG CCG CCG mFATP
M R A P G A G T A S V A S L A L L W F L L rFATP
ATG CCG GGT CCG GGT CCG GCG GCG TGG GTG GTC TGG CTG CCG CCG TGG TGG CCG CCG
M R **T** P G A G T A S V A S L **G** L L W **L** L L
ATG CCG **ACT** CCG GGA GGA GGA ACG GCG TGT GTG GCG TCA TGG GCG CCG CTT TGG CTT CCG

61 G L P W T W S A A A A **L** **G** V Y V G S G G hFATP1
GGG CTG CCG TGG ACC TGG AGC GCG GGA GCG GCG CTC GCG GCG TAC GCG GCG ACC GCG GCG mFATP
G L P W T W S A A A A F C V Y V G G G G rFATP
GGA CTT CCG TGG ACC TGG AGC GCG GCG GCG GCG TTC TGT GTG TAC GCG GGT GCG GCG GCG
G L P W T W S A A A A F **G** V Y V G S G G
GGA CTT CCG TGG ACC TGG AGC GCG GCG GCG GCG TTC GGT GTG TAC GCG GGT ACC GGT GCG

121 W R F L R I V C K T A R R D L F G L S V hFATP1
TGG CCG TTT CTG CCG ATC GTC TCC AAG ACC GCG AGG GGA GAC CCG TTT GGT CCG TGT GCG mFATP
W R F L R I V C K T A R R D L F G L S V rFATP
TGG CCG TTT CTG CCG ATC GTC TCC AAG ACC GCG AGG GGA GAC CCG TTT GGT CCG TGT GCG
W R F L R I V C K T A R R D L F G L S V
TGG CCG TTT CTG CCG ATC GTC TCC AAG ACC GCG AGG GGA GAC CCG TTT GGT CCG TGT GCG

181 L I R V R L E L R R H **Q** R A G **N** T I P **R** hFATP1
CTG ATC CCG GTG CCG CCG GAG CCG AGG CCG CAC CAG CCG GCG CCG AAC ACC ATC CCG CCG mFATP
L I R V R L E L R R H R R A G D T I P C rFATP
CTG ATC CCG GTT CCG CCG CTA GAG CCG GGA CCG CCG GCG GGA GGA GAC ACC ATC CCG CCG
L I R V R L E L R R H R R A G D T I P **R**
CTG ATC CCG GTG CCG CCG CTA GAG CCG GGA CCG CCG CCG GGA GGA GAC ACC ATC CCG CCG

241 I F Q A V **V** **Q** R Q P E R L A L V D A **G** **T** hFATP1
ATC TTT CAG GCG GGA GCG CAG CCA CAG CCG GCG CCG CTG CCG CCG GCG GAT CCG CCG ACC mFATP
I F Q A V A R R Q P E R L A L V D A S S rFATP
ATC TTT CAG GCG GGT GCG CCG CCG CCA CCA CCA GCG CCG CTG CCA CCG GCG GAC CCG AGT AGT
I F Q A V A **Q** R Q P E R L A L V D A S S
ATC TTT CAG GCG GCG GCG CAG CCA CAG CCG CCG CCG GCG GAT CCG AGT ACC

301 **E** C W T F A Q L D **A** Y S N A V A N L F hFATP1
GGC CAG TCC TGG ACC TTT GCG CAG CCG GCG GCG TAC TCC AAT CCG GGA GCG AAC CCG CCG mFATP
G I C W T F A Q L D T Y S N A V A N L F rFATP
GGT ATC TCC TGG ACC TTT CCA CAG CCG GCG ACC TAC TCC AAT CCG GGA GCG AAC CCG CCG
G I C W T F A Q L D T Y S N A V A N L F
GGT ATC TCC TGG ACC TTT CCA CAG CCG GCG ACC TAC TCC AAT CCG GTG GCG AAT CCG CCG

361 R Q L G F A P G D V V A **I** F L E G R P E hFATP1
CCG CAG CCG GCG TTT CCG CCG GCG GCG GCG GCG GCG TTT CCG GCG GCG CCG CCG mFATP
R Q L G F A P G D V V A V F L E G R P E rFATP
L Q L G F A P G D V V A V F L E G R P E
CTC CAG CCG GCG TTT CCG CCA GCG GAT GCG GCG GCG GCG TTT CCG GGA GCG CCG CCG CCG

421 F V G L W L G L A K A G **M** **E** A A L L N V hFATP1
TTC GTG CCG CTG TGG CCG GCG CCG ACC GCG GCG ATG GAG CCG CCG CTG CCG AAC CCG mFATP
F V G L W L G L A K A G V V A A L L N V rFATP
TTC GTG GGA CTG TGG CCG GCG CCG ACC GCG GCG GTG GCG CCG CTT CCG AAT CCG
F V G L W L G L A K A G V V A A L L N V
TTC GTG GGA CTG TGG CCG GCG CCG ACC GCG GCG GGA GTG GCG CCG CTT CCG AAT CCG

481 N L R R E P L A F C L G T S **G** A K A L I hFATP1
AAC CCG CCG CCG GAG CCG CCG GCG TTT TTT CCG GCG ACC TGG CCG CCG AAG GCG CCG ATC mFATP
N L R R E P L A F C L G T S A A K A L I rFATP
AAC CCG ACC CCG GAG CCG CCG GCG TTT TTT CCG GCG ACC TCA CCG CCG AAG GCG CCG ATT
N L R R E P L A F C L G T S A A K A L I
AAC CCG ACC CCG GAG CCG CCG GCG TTT TTT TGG GCG ACC TCA CCG CCG AAG GCG CCG ATT

541 **F** G G E M **V** A A V A E V S **G** **H** L G K S L hFATP1
TTT GGA GGA GAA ATG GCG CCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG mFATP
Y G G E M A A A V A E V S E Q L G K S L rFATP
TTT GCG GCG GAG ATG GGA CCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
Y G G E M A A A V A E V S E Q L G K S L
TTT GCG GCG GAG ATG GGA CCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG

601 I K F C S G D L G P E G I L P D T H L L hFATP1
AIC AAG TCC TCC TCT GGA GAC TTG GGG CCG GAG GGC ATC TTG CCG GAC ACC CAC CTC CCG
L K F C S G D L G P E S I L P D T Q L L mFATP
CTC AAG TCC TCC TCT GGA GAT CTG GGG CCG GAG ACC ATC CTG CCG GAC ACC CAC CTC CCG
L K F C S G D L G P E S V L P D T Q L L rFATP
CTC AAG TCC TCC TCT GGA GAT CTG GGG CCG GAG ACC ATC CTG CCG GAC ACC CAC CTC CCG

661 D P L L K E A S T A P L A Q I P S K G M hFATP1
GAC CCG CCG CTG AAG GAG GGC TCT ACT GTC CCG TTG GCA GAG AIC CCG ACC AAG GGC AAG
D P M L A E A P T T P L A Q A P G K G M mFATP
GAC CCG AAG CTT CTT GAG GGC CCG ACC ACA CCG CTG GCA CAA GTC CCA GGC AAG GGC AAG
D P M L A E A P T T P L A Q A P G K G M rFATP
GAC CCG AAG CTT CTT GAG GGC CCG ACC ACA CCG CTG GCA CAG GTC CCA GGC AAG GGC AAG

721 D D R L F Y L E Y S G L L G S L P K A A I hFATP1
GAC GAT CCG CTT TCC TAC AIC TAC AAG TTG GGG ACC ACC CCG CCG CCG AAG CCG CCG ATT
D D R L F Y T E Y S G L L G S L P K A A I mFATP
GAT GAT CCG CTT TTT TAC AIC TAT ACT TTG GGG ACC ACC CCG CCG CCG AAG CCG CCG ATT
D D R L F Y J A Y S G L L G S L P K A A I rFATP
GAT GAT CCG CTA TTT TAC AIC TAT ACT TCT GGG ACC ACC CCA CCG CCG AAG CCG CCG ATT

781 V V H S R Y Y R M A A F G H H A Y R M Q hFATP1
GTC GTC CAC ACC AGG TAC TAC CCG ATG GCA GGC TTC GGC CAC CAC GGC TAC CCG AIG CCG
V V H S R Y Y R I A A F G H H S Y S M R mFATP
GTC GTC CAC ACC AGG TAC TAC CCG ATT CCG GGC TTC GGC CAC CCG TCC TAC ACC AIG CCG
V V H S R Y Y R I A A F G H H S Y S M R rFATP
GTC GTC CAC ACC AGG TAC TAC CCG ATC GCA GGC TTC GGC CAC CCG TCC TAC ACC AIG CCG

841 A A D V L Y D C L P L Y H S A G N I I G hFATP1
GGC CCG GAC GTG CTC TAT GAC TCC CTG CCG CCG TAC CAC TCG CCA GGA AAC ATC AIC CCG
A A D V L Y D C L P L Y H S A G N I M G mFATP
GGC CCG GAT GTG CTC TAT GAC TCC CTG CCA CCG TAC CAC TCT CCA GGC AAC ATC AIG CCG
A N D V L Y D C L P L Y H S A G N I M G rFATP
GGC ACC GAT GTG CTC TAT GAC TCC CTA CCG CCG TAC CAC TCA CCA GGC AAC ATC AIG CCG

901 V G Q C L I Y G L T V V L R K K F S A S hFATP1
GTC GGC CCG TGT CTC AIC TAT GGC CTG ACA GTC GTC CTC CCG ACC AAA TTC TCG CCG ACC
V G Q C V I Y G L T V V L R K K F S A S mFATP
GTC GGC CCG TCC GTC AIC TAC GGC TTG AGG GTG GTA CTG CCG ACC AAG TTC TCC CCG ACC
V G Q C I I Y G L T V V L R K K F S A S rFATP
GTC GCA CCG TGT AIC AIC TAC GGC TTA AGG GTG GTA CTG CCG ACC AAG TTC TCC CCG ACC

961 R F W D D C I K Y N C T V V Q Y I G E I hFATP1
CCG TTC TCG GAC GAC TCT AIC AAG TAC APC TCC ACC GTG GTC CCG TAC ATC GGC GAG AIC
R F W D D C V K Y N C T V V Q Y I G E I mFATP
CCG TTC TCG GAT GAC TCT AIC AAG TAC AIT TCC ACC GTA GTG CCG TAC ATA GGT GAA AIC
R F W D D C V K Y N C T V V Q Y I G E I rFATP
CCG TTC TCG GAC GAC TCT GTC AAA TAT AIT TCC ACC GTA GTG CCG TAC ATC GGT GAA ATA

1021 C R Y L L K Q P V R E A E R R H R V R L hFATP1
CC CCG TPC CTG CTG AAG CAG CCG GTG CCG GAG GCG ACC CCA CAC CCG GTG CCG CCG
C R Y L L R Q P V R D V E Q R H R V R L mFATP
CC CCG TPC CTG CTG AAG CAG CCG GTC CCG GAG GCG ACC CCA CAC CCG GTG CCG CCG
C R Y L L R Q P V R D V E R R H R V R L rFATP
CC CCG TPC CTG CTA AAG CAG CCG GTC CCG GAT GTC GAG CCG CCG CAC CCG GTG CCG CCG

1081 A V G N G L R P A I W E E F T E R F G V hFATP1
GGC GTC GGC AAC GGC CTG CCG CCG CCG ATT TTG GAG GAG TTC AGG GAG CCG TTC CCG GCA
A V G N G L R P A I W E E F T Q R F G V mFATP
GGC GTC GGT AAT GGC CCG CCG CCG CCG ATT TTG GAG GAG TTC AGG CAG CCG TTC CCG GGC
A V G N G L R P A I W E E F T Q G F G V rFATP
GGC GTC GGT AAT GGC CCG CCG CCG CCG ATT TTG GAG GAG TTC AGG CAG CCG TTC CCG GGC

1141 R C I S E F Y G A T E C N C S I A N M D hFATP1
CCG CCA AAT GGC GAG TTT TAC CCG CCG CCG GAG TCG AAC TCC ACC AIT GGC AAC AIG GAC
P Q I S E F Y G A T E C N C S I A N M D mFATP
CCG CCA AAT GGC GAG TTT TAC CCG CCG CCG GAG TCG AAC TCC ACC AIT GGC AAC AIG GAC
R C I S E F Y G A T E C N C S I A N M D rFATP
CCG CCA AAT GGC GAG TTT TAC CCG CCG CCG GAG TCG AAC TCC ACC AIT GGC AAC AIG GAC

FIG. 2B

SUBSTITUTE SHEET (RULE 26)

1201G K V G S C G F N S R I L **P** H V Y P I R hFATP1
GGC AAG GTC GGC TCC TGT GGT TTC AAC ACC CGC ATC CTC CCC CAC GIG TAC CCC ATC CGC
G K V G S C G F N S R I L T H V Y P I R mFATP
GGC AAG GTC GGC TCC TGT GGT TTC AAC ACC CGT ATC CTC ACC CAC GIG TAC CCC ATC CGT
G K V G S C G F N S R I L T H V Y P I R rFATP
GGC AAG GTC GGC TCC TGT GGT TTC AAC ACC CGT ATC CTC ACC CAC GIG TAC CCC ATC CGT

1261L V K V N E D T M E **L** L R D **A** **O** G L C I hFATP1
CTG GTC AAG GTC AAT GAG GAC ACA ATG GAG CTG CTG CGG GAT CCG CAG GGC CTC TCC ATC
L V K V N E D T M E P L R D S E G L C I mFATP
CTG GTC AAG GTC AAT GAG GAC ACA ATG GAG CCA CTG CGG GAC TCC CAG GGC CTC TCC ATC
L V K V N E D T M E P L R D S **Q** G L C I rFATP
CTG GTC AAG GTC AAC GAG GAC AAG ATG GAG CCA CTG AAG GAC TCC CCA GGC CTC TCC ATC

1321P C Q A G E P G L L V G Q I N Q Q D P L hFATP1
CCC TCC CAG GGC CGG GAG CCT GGC CTC CTT GIG GGT CAG ATC AAC CAA CAG GAC CCG CIG
P C Q P G E P G L L V G Q I N Q Q D P L mFATP
CCC TCC CAG GGC CGG GAA CCT GGC CTT CIG GGC CAG ATC AAC CAG CAG GAC CCT CIG
P C Q P G E P G L L V G Q I N Q Q D P L rFATP
CCC TCC CAG GGC CGG GAA CCT GGC CTT CIG GGC CAG ATC AAC CAG CAA GAC CCT CIG

1381R R F D G Y V S **E** S A T **S** K K I A H S V hFATP1
GGC GGC TTT GAT GGC TAT GTT AGT GAG ACC GGC ACC AGC AAG AAG ATC GGC CAC AGC GTC
R R F D G Y V S D S A T N K K I A H S V mFATP
GGC GGT TTT GAT GGT TAT GTT AGT GAC AGT GGC ACC AAC AAG AAG ATT GGC CAC AGC GTC
R R F D G Y V S D S A T N K K I A H S V rFATP
GGC GGC TTT GAT GGC TAT GTT AGT GAC ACC GGC ACC AAC AAG AAG ATT GGC CAC AGC GTC

1441F **S** K G D S A Y L S G D V L V M D E L G hFATP1
TTC ACC AAG GGC GAC ACC GGC TAC CTC TCA GGT GAC GTG CTA GIG AIG GAT GAG CTG GGC
F R K G D S A Y L S G D V L V M D E L G mFATP
TTC CCA AAG GGC GAT ACC GGC TAC CTC TCA GGT GAC GTG CTA GIG AIG GAC GAG CTG GGC
F R K G D S A Y L S G D V L V M D E L G rFATP
TTC CCA AAG GGC GAC ACC GGC TAC CTT TCA GGT GAC GTG CTA GIG AIG GAC GAG CTG GGC

1501Y M Y F R D R S G D T F R W R G E **N** V S hFATP1
TAC ATG TAC TTC CCG GAC GGT ACC GGG GAC ACC TTC CCG TGG CCA GGC GAG AAC GTC TCC
Y M Y F R D R S G D T F R W R G E N V S mFATP
TAC ATG TAT TTC CCG GAC GGT ACC GGG GAC ACC TTC CCG TGG CCG GGC GAG AAC GTC TCC
Y M Y F R D R S G D T F R W R G E N V S rFATP
TAC ATG TAC TTC CCG GAC GGT ACC GGG GAT ACC TTC CCA TGG CCG GGC GAG AAC GTC TCC

1561 **N** T E V E **G** V L S R L L G Q T D V A V Y hFATP1
AAC ACC GAG GTC GAG GGC GTC CTG AGC CCG CTG CTG GGC CAG ACA GGC GTG GGC GTC TAT
T T E V E A V L S R L L G Q T D V A V Y mFATP
ACC ACC GAG GTC GAA GGC GTC CTG AGC CCG CTA CTG GGC CAG ACC GGC GTG GGT GTC TAT
T T E V E A V L S R L L G Q T D V A V Y rFATP
AAC ACC GAG GTC GAA GGC GTC CTG AGC CCG CTG TTG GGC CAG ACC GGC GTG GGT GTC TAT

1621G V A V P G V E G K A G M A A **V** A D P H hFATP1
GGG GIG GCT GTT CCA GGA GIG GAG GGT AAG GGA GGG ATG GCG GGC GTC GCA GAT CCC CAC
G V A V P G V E G K A G M A A I A D P H mFATP
GGG GIG GCT GTT CCA GGA GIG GAG GGT AAG GGT GGC ATG GCA GGC ATC GCA GAT CCC CAC
G V A V P G V E G K S G M A A I A D P H rFATP
GGG GIG GCT GTT CCA GGA GIG GAG GGT AAG ACC GGC ATG GCG GGC ACT GCA GAT CCC CAC

1681S **L** L D P N **A** **I** Y Q E L Q K V L A **P** Y A hFATP1
AGC CTGTG GAC CCC AAC GGC ACA TAC CAG GAG CTG CAG AAG GIG CTG GCA CCA TAT CCG
S Q L D P N S M Y Q E L Q K V L A S Y A mFATP
AGC CAG TIG GAC CCA AAC TCA AGC TAC CAG GAA TTA CAG AAG GGT CTT CCA TCC TAT CCG
N Q L D P N S M Y Q E L Q K V L A S Y A rFATP
AAC CAG CTG GAC CCA AAC TCA AGC TAC CAG GAA TTG CAG AAG GGT CTT CCA TCC TAT CCG

1741 R P I F L R L L P Q V D T T G T F H I Q hFATP1
GGG CCC ATC TTC CTG GGC CTT CCG CCC CAG GIG GAC ACC ACA GGC ACC TTC AAG ATC CCG
R P I F L R L L P Q V D T T G T F H I Q mFATP
GGG CCC ATC TTC CTG GGC CTT CCG CCC CAG GIG GAT ACC ACA GGC ACC TTC AAG ATC CCG
Q P I F L R L L P Q V D T T G T F H I Q rFATP
CAG CCC ATC TTC CTG GGC CTT CCG CCC CCA GIG GAT ACA ACA GGC ACC TTC AAG ATC CCG

FIG. 2C

SUBSTITUTE SHEET (RULE 26)

1801 K T R L Q R E G F D P R Q T S D R L F F hFATP1
 AAG ACG ACG CTG CAG CGA GAG GGC TTT GAC CCA CG CAG ACC TCA GAC CGG CTC TTC TTC
 K T R L Q R E G F D P R Q T S D R L F F mFATP
 AAG ACC CCG CTG CAG CGT GAA GGC TTT GAC CCG CAG ACC TCA GAC AGG CTC TTC TTT
 K T R L Q R E G F D P R Q T S D R L F F rFATP
 AAG ACC CCA CTA CAG CGT GAA GGC TTT GAC CCG CAG ACC TCA GAC CGG CTC TTC TTT

1861 L D L K Q G **H** Y **L** P L **N** E **A** V **Y** **T** R I C hFATP1
 CTG GAC CCG AAG CAG GGC GAC TAC CTG CCG TTA AAT GAG CCA GGC TAC ACT CCG ATC TCC
 L D L K Q G R Y V P L D E R V H A R I C
 CTA GAC CCG AAG CAG GGA GGC TAT GGA CCG CTG GAT GAG AGA GGC CAT GCC CCG ATT TCT mFATP
 L D L K Q G R Y **L** P L D E R V H A R I C
 CTA GAC CCG AAA CAG GGA GGC TAC CTA CCG CTG GAT GAG AGA GGC CAT GCC CCG ATC TCC rFATP

1921 **S** G **A** F **A** L .
 TCG GGC GGC TTC CCG CCG TGA hFATP1
 A G D F S L .
 GCA GGC GAC TTC TCA CTC TGA mFATP
 A G D F S L .
 GCA GGC GAC TTC TCA CTC TGA rFATP

FIG. 2D

FIG. 3

hsFATP1 cDNA

						Start Codon	
CGGGGAGACG	GGACGTGAAG	<u>GGATG</u> CGGGC	TCCTGGAGCA	GGAACAGCCT	CTGTGGCCTC	60	
ACTGGCGCTG	CTTTGGTTTC	TGGGACTTCC	GTGGACCTGG	AGCGCGGGCG	CGGCGTTCTG	120	
TGTGTACGTG	GGTGGCGGCG	GCTGGCGCTT	TCTGCGTATC	GTCTGCAAGA	CGGCGAGGCG	180	
AGACCTCTTT	GGCCTCTCTG	TTCTGATTCT	TGTTCCGGCTA	GAGCTGCGAC	GACACCGGCG	240	
AGCAGGAGAC	ACGATCCCCG	GCATCTTCCA	GGCTGTGGCC	CGGCGACAAC	CAGAGCGCCT	300	
GGCACTGGTG	GACGCCAGTA	GTGGTATATG	CTGGACCTTC	GCACAGCTGG	ACACCTACTC	360	
CAATGCTGTA	GCCAACCTGT	TCCGCCAGCT	GGGCTTTGCA	CCAGGCGATG	TGGTGGCTGT	420	
GTTCTTGAG	GGCCGGCCGG	AGTTCGTGGG	ACTGTGGCTG	GGCCTGGCCA	AGGCCGGTGT	480	
GGTGGCTGCT	CTTCTCAATG	TCAACCTGAG	GCGGGAGCCC	CTGGCCTTCT	GCCTGGGCAC	540	
ATCAGCTGCC	AAGGCCCTCA	TTTATGGCGG	GGAGATGGCA	GCGGCGGTGG	CGGAGGTGAG	600	
CGAGCAGCTG	GGGAAGAGCC	TCCTCAAGTT	CTGCTCTGGA	GATCTGGGGC	CTGAGAGCAT	660	
CCTGCCTGAC	ACGCAGCTCC	TGGACCCCAT	GCTTGCTGAG	GCGCCACCA	CACCCCTGGC	720	
ACAAGCCCCA	GGCAAGGGCA	TGGATGATCG	GCTGTTTTAC	ATCTATACTT	CTGGGACCAC	780	
CGGGCTTCCT	AAGGCTGCCA	TTGTGGTGCA	CAGCAGGTAC	TACCGCATTG	CTGCCTTTGG	840	
CCACCATTCC	TACAGCATGC	GTGCCGCCGA	TGTGCTCTAT	GA CTGCCTGC	CACTCTACCA	900	
CTCTGCAGGG	AACATCATGG	GTGTGGGGCA	GTGCGTCATC	TACGGGTTGA	CGGTGGTACT	960	
GCGCAAGAAG	TTCTCCGCCA	GCCGCTTCTG	GGATGACTGT	GTCAAGTACA	ATTGCACGGT	1020	
AGTGCAGTAC	ATAGGTGAAA	TCTGCCGCTA	CCTGCTGAGG	CAGCCGGTTC	GCGACGTGGA	1080	
GCAGCGACAC	CGCGTGCGCC	TGGCCGTGGG	TAATGGGCTG	CGGCCAGCCA	TCTGGGAGGA	1140	
GTTCACGCAG	CGCTTCGGTG	TGCCACAGAT	CGGCGAGTTC	TACGGCGCTA	CCGAGTGCAA	1200	
CTGCAGCATT	GCCAACATGG	ACGGCAAGGT	CGGCTCCTGC	GGCTTCAACA	GCCGTATCCT	1260	
CACGCATGTG	TACCCCATCC	GTCTGGTCAA	GGTCAATGAG	GACACGATGG	AGCCACTGCG	1320	
GGACTCCGAG	GGCCTCTGCA	TCCCGTGCCA	GCCCGGGGAA	CCCGGCCTTC	TCGTGGGCCA	1380	
GATCAACCAG	CAGGACCCCT	TGCGGCGTTT	CGATGGTTAT	GTTAGTGACA	GTGCCACCAA	1440	
CAAGAAGATT	GCCCACAGCG	TTTTCCGAAA	GGGCGATAGC	GCCTACCTCT	CAGGTGACGT	1500	
GCTAGTGATG	GACGAGCTGG	GCTACATGTA	TTTCCGTGAC	CGCAGCGGGG	ACACCTTCCG	1560	
CTGGCGCGGG	GAGAACGTGT	CCACCACGGA	GGTGGAAGCC	GTGCTGAGCC	GCCTACTGGG	1620	
CCAGACGGAC	GTGGCTGTGT	ATGGGGTGCC	TGTGCCAGGA	GTGGAGGGGA	AAGCTGGCAT	1680	
GGCAGCCATC	GCAGATCCCC	ACAGCCAGTT	GGACCCTAAC	TCAATGTACC	AGGAATTACA	1740	
GAAGGTTCTT	GCATCCTATG	CTCGGCCCAT	CTTCCTGCGT	CTTCTGCCCC	AGGTGGATAC	1800	
CACAGGCACC	TTCAAGATCC	AGAAGACCCG	GCTGCAGCGT	GAAGGCTTTG	ACCCCCGTCA	1860	
GACCTCAGAC	AGGCTCTTCT	TTCTAGACCT	GAAGCAGGGA	CGCTATGTAC	CCCTGGATGA	1920	
GAGAGTCCAT	GCCCGCATT	GTGCAGGCGA	CTTCTCACTC	<u>TGA</u> AGCTGTT	CCTCTACTGG	1980	
CCACAAACTC	TGGGCGTGGT	GGGAGAGGCC	AGCTTGAGCC	AGACAGCGCT	GCCCAGGGGT	2040	
GGCCGCCTAG	TACACACCCA	CCTGGCCGAG	CTGTACCTGG	CACGGCCCAT	CCTGGACTGA	2100	
GAAACTGGAA	CCTCAGAGGA	ACCCGTGCCT	CTCTGCTGCC	TTGGTGCCCC	TGTGTCTGCC	2160	
TCCTCTCCCT	GCTTTTCAGC	CTNTGTCTCC	TTCCATCCNT	GTCCCTGTNT	GGCCTTAACC	2220	
CG						2222	

Stop Codon

hs FATP1

FIG. 4A

ATGCGGGCTC	CGGGTGCGGG	CGCGGGCCTCG	GTGGTCTCGC	TGGCGCTGTT	GTGGCTGCTG	60
GGGCTGCCCCT	GGACCTGGAG	CGCGGGCAGCG	GCGCTCGGGCG	TGTACGTGGG	CAGCGGGCGGC	120
TGGCGCTTCC	TGCGCATCGT	CTGCAAGACC	GCGAGGCGAG	ACCTCTTGTTG	AGTGTTGCGG	180
GATCCAGGGC	TGGGGGCGGG	GCTGAGGGCT	CTGGGGGGCCC	ACGCTGCAGG	GCTGGGCTTG	240
CGGGAGGCCCT	TGGAGGTGGA	GAGTGAXXXX	XXXXXATACG	CTGAGATCTA	CTCTCTGCTG	300
TGTAATGCTG	CCTGGTCACT	GAGAGATCAG	CACAAAGTTC	ACATCGCCTC	ATGAAAGCCT	360
GCTGCCTGGG	TCTCAGCGGG	AGGCTGAGGC	TCCAGAGGCC	AGGCGGGGCA	GGGCACCACT	420
GACGCTGTCC	CTCCGTCCCTC	CCTCCCAGCG	GTCTCTCTGT	GCTGATCCGC	GTGCGCCTGG	480
AGCTGCGGGG	CCACCAGCGT	GCCGGCCACG	GTCTCTCTGT	GCTGATCCGC	GTGCGCCTGG	540
AGCTGCGGGG	GCACCAGCGT	GCCGGCCACA	CCATCCCAGC	CATCTTTCAG	GCGGTAGTGC	600
AGCGACAGCC	CGAGCGCCTG	GCGCTGGTGG	ATGCCGGGAC	CGGCGAGTGC	TGGACCTTTG	660
CGCAGCTGGA	CGCTACTCC	AATGCGGTAG	CCAACCTCTT	CCGCCAGCTG	GGCTTCGCGC	720
CGGGCGACGT	GGTGGCCATC	TTCCTGGAGG	GCCGGCCGGA	GTTCTGTGGG	CTGTGGCTGG	780
GCCTGGCCAA	GGCGGGCATG	GAGGCCGCGC	TGCTCAACGT	GAACCTGCGG	CGCGAGCCCC	840
TGGCCTTCTG	CCTGGGCACC	TCGGGCGCTA	AGGCCCTGAT	CTTTGGAGGA	GAAATGGTGG	900
CGGGTGAGGC	CAGGCGTGGG	CATCAGGTGG	GCGGGGACCC	AGGACTGGCC	CCTGGGCGGG	960
CGGGGAGTCT	GCTGCGCCCC	AGGCCTCGGA	AGGCGGCCGC	CTGGACGTGG	GCATGAGGTG	1020
CACGGTCTGG	GTATGCCCCG	GGCAGGGAGT	TGGTGATCC	CAGGCCTCGG	GAGGGGCTTG	1080
TCCGGCGGTG	ACCATGACCC	ATGTGTTGGG	GACCACAGCG	GTGGCCGAAG	TGAGGGGCCA	1140
TCTGGGGAAA	AGTTTGATCA	AGTTCTGCTC	TGGAGACTTG	GGGCCCCGAG	GCATCTTGCC	1200
GGACACCCAC	GTCCTGGACC	CGCTGCTGAA	TGGAGCCTCT	ACTGCCCCCT	TGGCACAGAT	1260
CCCCAGCAAG	GTCATGGACG	GTGAGTCAAC	TTCCAGGACA	CCTCTACCCC	AATGACTCAG	1320
GCCCCACCCC	CTAACACTGT	ATCTCCTGCA	GATCGTGTTT	TCTAGATCTA	GAEGTGGGGG	1380
ACCACCGGGC	TGCCCAAGGC	TGCCATTGTC	GTGCACAGCA	GGTGAGGGGC	CCACAGGCAT	1440
AATGCCCTCA	GCCGCTGAGA	GTGACCCAGG	CATCTTGCCA	GCCTGACCTG	CCCCTCAGCT	1500
CCTGTGGGCA	TCTCCATGTT	ACCCTGGGGA	CAGAGAGGGC	AGCTGGTGTT	TCCTGAGCAC	1560
TTGCTCTGTG	TCCAGACCAG	GGCCAAGCCC	TGGACGTGTA	AACTCATTGC	AAGGGTCATA	1620
ACTGCTTGGG	GCTGGACAAA	GGCATCACAC	CATTTTTCACA	CCATCTTGGG	ACCXXXXXXX	1680
XXXXXXXXXGG	GGATTTAGGT	CCAGCCTCTG	CCTCCGGCTC	CCCCTCCCCC	TGCAGGTACT	1740
ACCGCATGGC	AGCCTTCGGC	CACCACGCCT	ACCGCATGCA	GGCGGCTGAC	GTGCTCTATG	1800
ACTGCCTGCC	CCTGTACCAC	TCGGCAGGTA	CTACGGCCTG	GGTAGGGAAT	GGTGGGTGGG	1860
GGCGGGGGAC	CCCTTACCAA	GGCCACCCTC	TGCAGGAAAC	ATCATCGGCG	TGGGGCAGTG	1920
TCTCATCTAT	GGGCTGACAG	TCGTCTCTCC	CAAGAAATTC	TCGGCCAGCC	GCTTCTGGGA	1980
CGACTGCATC	AAGTACAAC	GCACGCCTTG	GCNNTCCGAA	AGTGCTGAGA	GNACAGGCGT	2040
GAGCTTTGTG	CCCGGCCAGG	GCCCAGCCCT	TTTATCGGTG	CAGCTGCTGG	CATCCCCTGC	2100
TGCAAGCTTC	CAGCTTCCTT	ATTTGTGTTT	GCAGCCCAAT	CTTCCAGGCT	GTTCTTTGTT	2160
AGAAAATAAG	TGATTTCTTG	GGCTGCTTTT	TGTTAGAANN	GAAGTTCTAC	CGAGGGTNNC	2220
NTGTTTCCAA	AAAAAAAAAA	TAGGNAAATT	NGATTAGATA	CTNACTNGCA	TAATANGNGA	2280
CNGNGTTGGG	NNCTTCCANA	GATTTGCGCC	CCTATGCGGA	TGGCACATCT	GTCCTCACC	2340
GGTTTTTCAC	GGTCTATTTA	GATCAGATAC	AGCCTCCCTG	NTAGCTTGTN	TGTAAAAATC	2400
CTTGCTTTTC	TTTTTTTTTC	TGAGGCGGAG	TCTCACTCTT	GTCCCCAGGC	TGGAGTACAA	2460
TAGAGCGATC	TTGGCTTACT	GCAACCTCTG	CCTCTCGGGT	TCAAGAGATT	CTCCTGCCTC	2520
AGCCTCCCAA	GTAGCTGGGA	TTACAGGCAC	ATGCCACCAG	GTCTGGCAAA	ATTTTGTGTT	2580
ATTTTCTTAG	AGATGAGGTA	TTACTACATT	GGNTAGGCTG	GTCTCGAACT	TCTGACCTGA	2640
NGTGATCTGC	CCACCTNGGN	CCCCCCAAAG	TGCTGGGANN	ACAGGCATGA	GCCACGGNGC	2700
CCAGCCATGT	GCCANTTTT	TTTTTTTTTT	TTGAGATGGA	GTCTNGGTNT	TGTCGCCCCA	2760
GGCTGGAGTG	CCATTGGGCG	AANTTGGNCC	AATTNNAGC	TCCGCCTCCC	GGGTNTACGC	2820
CATTCTCTTG	CCTNAGCCTC	CCGAGTAGCT	GGGATTACAG	GAGTGTGCCA	CCACGNCCTG	2880

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FIG. 4B

NTAATTTTTT	GNATTTTTTTA	GTAGAGACGG	GGTTTNACCA	TGTTAGCCAG	GATGGTCTCG	2940
ATCTCCTGAC	NTNGTGATCC	ACCTGCTTTG	NCNTCCCCAA	GTGCTGGGAT	NACAGGCTTG	3000
AGCCACCCCG	CCTGGCCGTG	CCCATTCTTA	AGCCAGTCAT	ATTGGCNTGA	GGAGGGGCTG	3060
GGGTGATTTN	CCAGGTTNTG	TCGGTAGAGA	TNTTGTTGGG	TGAACCANAC	CCTAAGGCAG	3120
TGGCCCAATT	NTGNGAATGG	GGGNGGGGAC	CTTCCCCCCC	TNNGACCAGG	TGTTTGTCT	3180
GCAGCCAGTG	ATCTTGGGTG	CATGCATTTC	TTNTCTGGGC	TTCAGTGCTT	CATCTCCAAG	3240
NTCAGCTTCA	GAGCATGGCG	GTGGTGTCTG	CTGATCATAT	TGGTTTAGTC	ATCNGCENNNT	3300
TNGTCAGNAC	NNTCTTCTGG	GGGANNAAGG	GGGNNCAGGG	GTGCTCTCAG	TGACGTTTGA	3360
GCTGAGACTG	AAGAGTTCAG	GCAAANGCAT	CTGGGGGAAG	ACTGCTTCAG	GCAAAAGGCT	3420
AGCATGTGCA	AAGTCCCTGA	GGAGGCAGAG	TGTTTCAGCGG	AGATGGAGGG	AGAGGAGGCA	3480
GCAGGGGCGG	CAGGGCCAGA	TCCAGTTGGC	CACAGGTAGA	AATGTGGGTT	TCATCCTGTG	3540
TGTGAGCGGG	AAGCCCCGAG	CACANTTGCA	GCTGNTATGA	TCCCATCTGG	TGGGTAATCC	3600
CCTGGCANGC	NANGGNTTCA	TTCCCATTNT	GGGGGCCGGG	ATGCCTGGGT	TCAAATCAAA	3660
TTCTGCCACT	TCCCAGCTCN	GTGATCTCGT	TCCAGTTCCT	TACCATCTGT	GAGCCTCGGT	3720
TTCTCCATCT	GTAAAGTGGC	GGGGGGGGAG	TCATTTCCGA	AGCCCAACTG	GAGATGAGAA	3780
NTNTTAAAGG	CACCGTGGGG	CACCTAGTGG	ATGACCCAG	GAAGNTCTTG	CGGGGCTTGA	3840
GAATGTGGCA	GGGAGGGAAG	ATGTCCTGCA	GGGGGAGCCC	ATGAGCAGGT	GGCTCCGTGG	3900
GCACCTGGGG	NTCAGTCCAG	ACGCAGCGCA	GCCTAGTGGG	TGCACCTGGG	AGTGGTCTGC	3960
CCAGGGGTGA	CGCTCCGGCC	AGCCCAGGCT	CAGCCAGGGG	GCTCTGGGTG	ACAGTGTAGG	4020
CTCCAGGCTG	AGNGTTGCCT	TTNGGGGTGC	CAGNTCTCCT	GCTAGTCTTG	CCCTGTGTGC	4080
CTGCTGAGGC	TCAGACAGGG	AGTGGTATCT	CCAGGAAGCT	GCTTGGCAGA	ATCGAGGGAC	4140
CACGAAGGGG	TGCGGGTACG	GGGAGGAGGA	AAGACACTGC	ATCTGCTTGG	ATAGAAGTTT	4200
GCATCCGGCC	AGGCACAAGT	GGCTCACACC	TGGAATCTCA	CACTTTGGGA	GGCTGAGGCA	4260
GGAGGATTGC	TTGAGGGCAG	GAGTTCAAGA	CCAGGCTGGG	CAACAGAGGG	AGATGGCCGA	4320
TCTGTACAAA	AAAATCTGTA	GTCCCAGCTA	CTCGGGAGGC	TAAGGCTGGA	GGATCACTTG	4380
AAGGCACCCC	GTTAGAGGCT	GCAGTGAGCT	GTGATCATGC	AACTGCATNC	AGCTNNGGGG	4440
GGGCAGGGGG	AGACCCCCCC	CCNAAAAACA	ACAAAAAAG	CTGCATCNTA	GACCCTTTGC	4500
AAGAGACTGA	ACGAGTCCTA	GGAGTCAATG	TGGTCCCTAA	TGGAGTGTGG	ANGATTCTGC	4560
AGCCATCATC	ATCCTTAGGC	TGTTCCGCTC	ATAGGATTAG	CTCCCTGGGT	GGGGCGGTCT	4620
CGGGGTNTCT	ACCTCTGATC	CGGGCTCCCC	ACCGCCTGCC	GGTCCCATCA	CCCACTTCCT	4680
CACCCCGTCC	CCCAGGTGGT	TCAGTACATC	GGGGAGATCT	GCCGCTACCT	GCTGAAGCAG	4740
CCGGTGCGCG	AGGCGGAGAG	GCGACACCGC	GTGCGCCTGG	CGGTGGGGAA	CGGGCTGCGT	4800
CCTGCCATCT	GGGAGGAGTT	CACGGAGCGC	TTCGGCGTAC	GCCAAATCGG	GGAGTTCTAC	4860
GGCGCCACCG	AGTGCAACTG	CAGCATTGCC	AACATGGACG	GCAAGGTGCA	CACCGGCAGG	4920
CCCCGGGGCA	GGTCTCGGAG	TTCAGGGAAG	ACACTTGTCT	CCTCTTCCTG	GGCCCTGGAT	4980
ACATAAAACA	GCCTGGACTG	GCGCGGAAGG	CTCGCAAGGC	GCACGCAGGG	CGGTGTAGGA	5040
GATCTGGACT	CCGTGCACAC	AGAACTCTGA	GXXXXXXXGAC	XXXXXXXGAC	TAGCTGTAGG	5100
TTCACACAGA	GGATCTACTT	CCTGCCAGAC	TGAGGTTTGT	GGATCAGGAA	GTGGCACCAG	5160
CCAGAGGTCT	TCTCTTGACT	GAATGCAGGC	TGGGAAGGTG	GGAGGAGGGG	GCCTGAGTTG	5220
GAGGCGACGC	TTACTACCCT	GCTTTTGCAG	ATCAGCCAAG	GCAGGCAAAG	TGTTATGAGA	5280
AGGACCCCGC	ATATCCCCGG	CTTTCCTACT	CAGTTCACCC	CATTCCAGGT	CGGCTCCTGT	5340
GGTTTCAACA	GCCGCATCCT	GCCCCACGTG	TACCCCATCC	GGCTGGTGAA	GGTCAATGAG	5400
GACACAATGG	AGCTGCTGCG	GGATGCCCCAG	GGCCTCTGCA	TCCCCTGCCA	GGCCGGGGTG	5460
AGCAGGGCCC	CCGCATGGTC	CCCACCCGGA	GCAGGGGTCC	CCACACCCTG	CCTGCCTAGC	5520
GCAGCCTGAA	CATGGCCTTC	TCCCTAGGGG	AGCCTGGCCT	CCTTGTGGGT	CAGATCAACC	5580
AACAGGACCC	GCTGCGCCGC	TTCGATGGCT	ATGTCAGCGA	GAGCGCCACC	AGCAAGAAGA	5640
TCGCCCCACAG	CGTCTTCAGC	AAGGGCGACA	GCGCCTACCT	CTCAGGTGCG	CAGCTGCTAG	5700
GCCCCGGTGA	CTGGCTGTGC	GGATGGGGAT	CGGCCACCCA	TTTGCCCCCTC	TCCCCTCTGC	5760
CAGGTGACCT	GCTAGTGATG	GATGAGCTGG	GCTACATSTA	CTTCCGGGAC	CGTAGCGGGG	5820

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FIG. 4C

ACACCTTCCG	CTGGCGAGGG	GAGAACGTCT	CCACCACCGA	GGTGGAGGGC	GTGCTGAGCC	5880
GCCTGCTGGG	CCAGACAGAC	GTGGCCGTCT	ATGGGGTGGC	TGTTCCAGGC	AAGCTGGGGT	5940
TGCAGGGGGT	GGTCCTGAGG	CATGGTCCTG	AGGGAGCTCA	GCCAAAAGGG	GCTTAGAGGT	6000
ACACATGCCT	TTGGCAGTGC	ACAACCTGGA	CAACTGCTCA	TGGCAGCCCA	GGAGGAAGCA	6060
CTGGATCTGG	AGCCAGTTCA	CCTGGGTGAT	GTTGAGCCTC	AGTTTTGTCA	TCAGAAAATG	6120
GGATCATGAA	AGCCCACCTG	TATTAGGGCT	TCAATGAGCC	AAGCAGGAGC	TCCCCAAAAT	6180
GTGTGGCTGC	TTCCATAAAT	GTCATCCCAG	GTTGGGAGAG	ACTGGAGATT	ACAGACCTGC	6240
TACTGCTTGA	CAGTGTATCT	GGTCCTGCTG	GTGAGGATGA	GAGGCGGGGT	GTCCTCAGCT	6300
CCTCTGCCTC	CAGGAGTGGA	GGGTAAGGCA	GGGATGGCGG	CCGTCGCAGA	CCCCCACAGC	6360
CTGCTGGACC	CCAACGCCAT	ATACCAGGAG	CTGCAGAAGG	TGCTGGCACC	CTATGCCCCG	6420
CCCATCTTCC	TGCGCCTCCT	GCCCCAGGTG	GACACCACAG	GTGCGAGTCT	CCCCCACTCC	6480
AACCTCTCTC	TTCATCCATC	AGTGTGTCTG	TTGATTCGAG	GGATATTGAG	TTGAGGCCTC	6540
CAGAAGCCAC	CTGCTCAGCC	CTTATCTGCC	CCCCATCCCC	ACTATAGGCA	CCTTCAAGAT	6600
CCAGAAGACG	AGGCTGCAGC	GAGAGGGCTT	TGACCCACGC	CAGACCTCAG	ACCGGCTCTT	6660
CTTCCTGGAC	CTGAAGCAGG	GCCACTACCT	GCCCTTAAAT	GAGGCAGTCT	ACACTCGCAT	6720
CTGCTCGGGC	GCCTTCGCCC	<u>TCTGA</u>		6745		

13 / 32

MRAIAGAAVVSLALLWLLGLPWTWSAAAAALGVYVSGGWRFLRIVCKTARRDLFGLSVLIRVRLELRRHRAGHTIPRIFQAVVQRPRLALVDAGT 100
GEXWTFQAQLDAYSNAPANLFRQLGFAPGDVVAIFLEGRPEFVGLWLGLAKAGMEAAALLNVNLRREPLAFCLGTSGAKALIFGEMVAAVAESGHLGKSL 200
IKFCSGDLGPEGILPDTHLLDPLLKEASTAPLAQIPSKGMDRLFYIYTSGTTGLPKAAIVVHSRYRMAAFGHHAYRMAQADVLYDCLPI.YHSAGNIIG 300
VGGCTI.YGLTVVLRKKFSASRFWDDCIKYNCTVVQYIGEICRYLLKQPVREAERRHRVRLAVGNGLRPAIWEETFTERFGVRQIGEFYGATECNCNCSIANMD 400
GKVAERCGFNRILPHIVYPIRIVKVNEDTMELL.RDAQGLCTPCQAGEPGLLVGQINQQDPLRRFDGCVSESATSKKIAHSVFSKGSAYLSGDVIVMDLIG 500
THHFEDESGHDTFRWRGHHVSNTEVEGVLSRLLGQTDVAVYGVAVPGVEGKAGMAA VADPHSLDPNAIYQELQKVLAPYARPIFLRLLPQVITTTGTFKIQ 600
KTFADREGGFDRQTSDRLEFFLDLKQGHYLPPLNEAVYTRICSGAFAL. 647

FIG. 5

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murine FATP

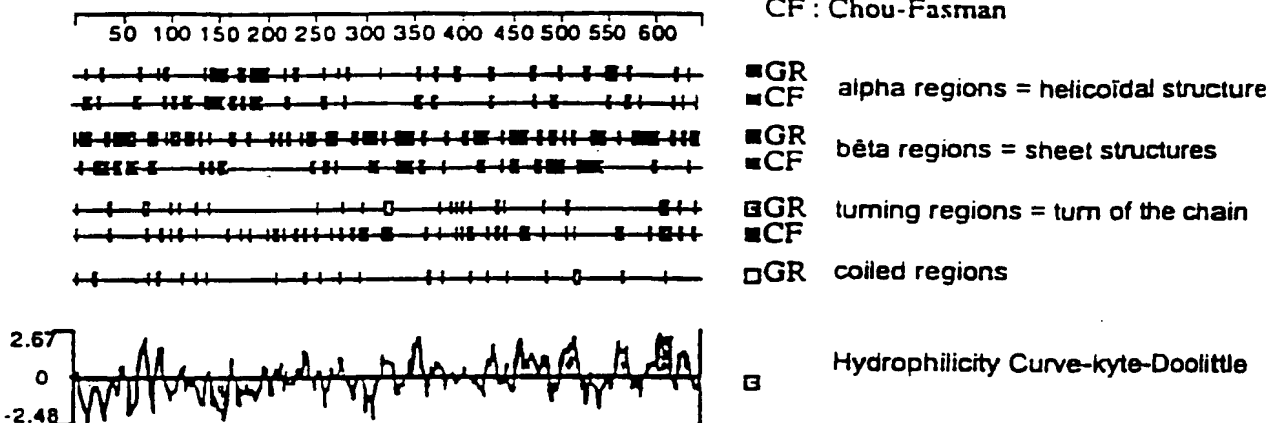


FIGURE 6A

human FATP

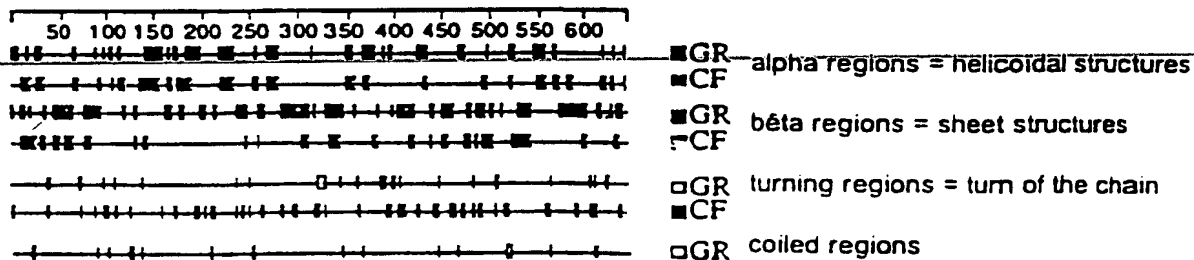


FIG. 6B

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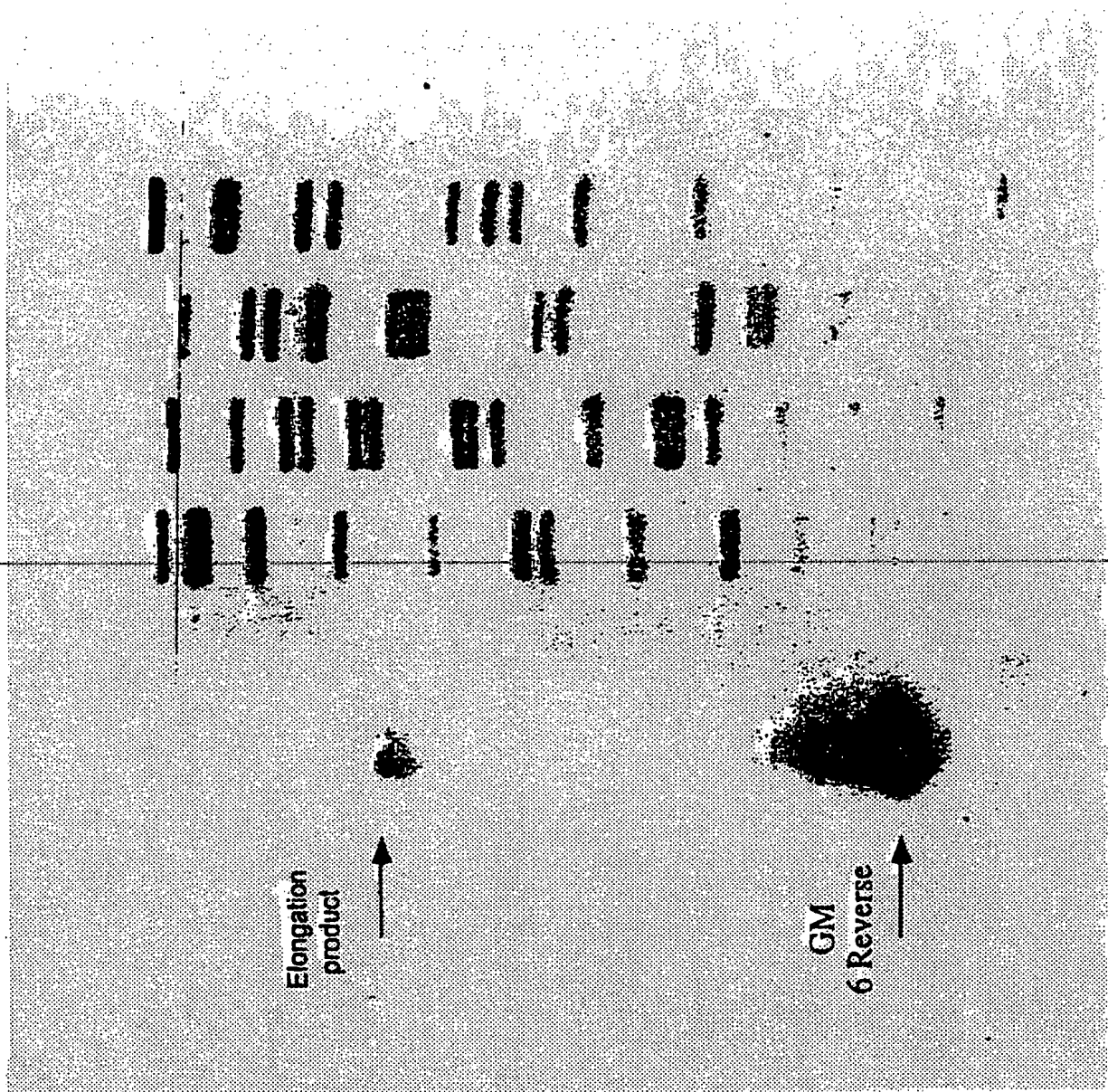


FIG. 7

SUBSTITUTE SHEET (RULE 26)

FIG. 8A

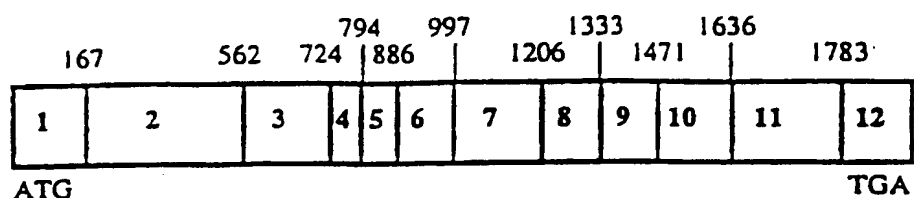


FIG. 8B

GGCGAGACCTCTT--- Intron 1 ? ---CGGTCTCTCTGT
 AAATGGTGGCGGgtgaggcc---215---gaccacagCGGTGGCCGAAG
 AGGGCATGGACGgtgagtca----131----tctgcagATCGTCTTTTCT
 GGTGGAGAGGAGgtgagggg---1500---eeeetgeagGTACTAGGGGAT
 ACCACTCGGCAGgttactacg-----68-----ctctgcagGAAACATCATCG
 TACAACTGCACG ----->3kb-----cgtccccacGTCGGCTCCTGT
 TGGACGGCAAGgtgcacacc---3000---cattccagGTCGGCTCCTGT
 CCCTGCCAGGCCgtgagcag---2900---ctccctagGGGAGCCTGGCC
 CCTACCTCTCAGgtgcgcag-----90----tctgccagGTGACGTGCTAG
 TGGCTGTTCCAGgtcaagct---389---gcctccagGAGTGGAGGGTA
 TGGACACCACAGgtgcgagt---2800---cactatagGCACCTTCAAGA

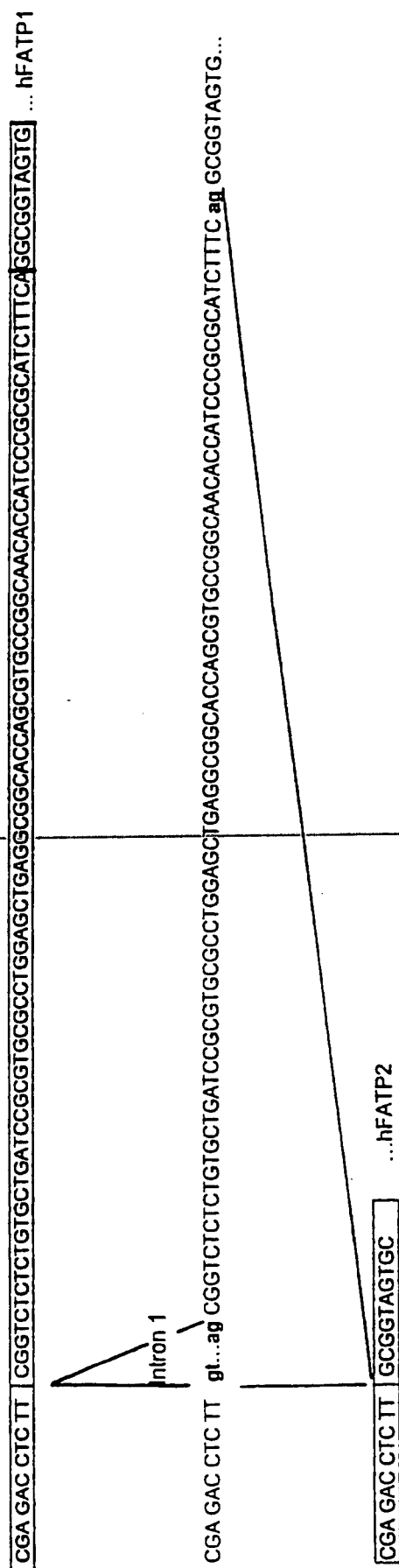


FIG. 8C

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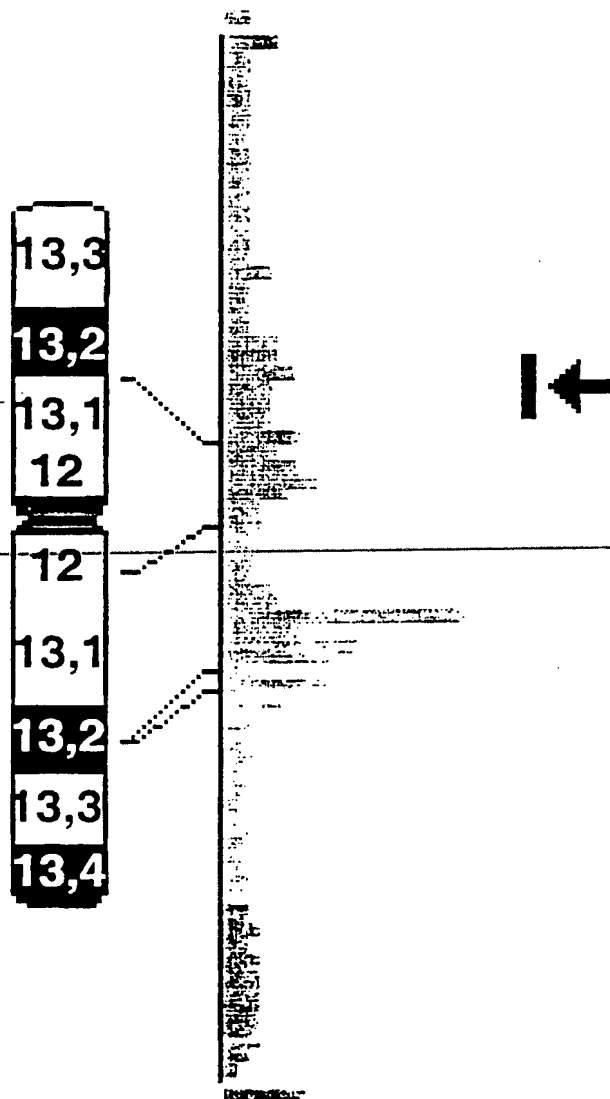


FIG. 9

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A FAO

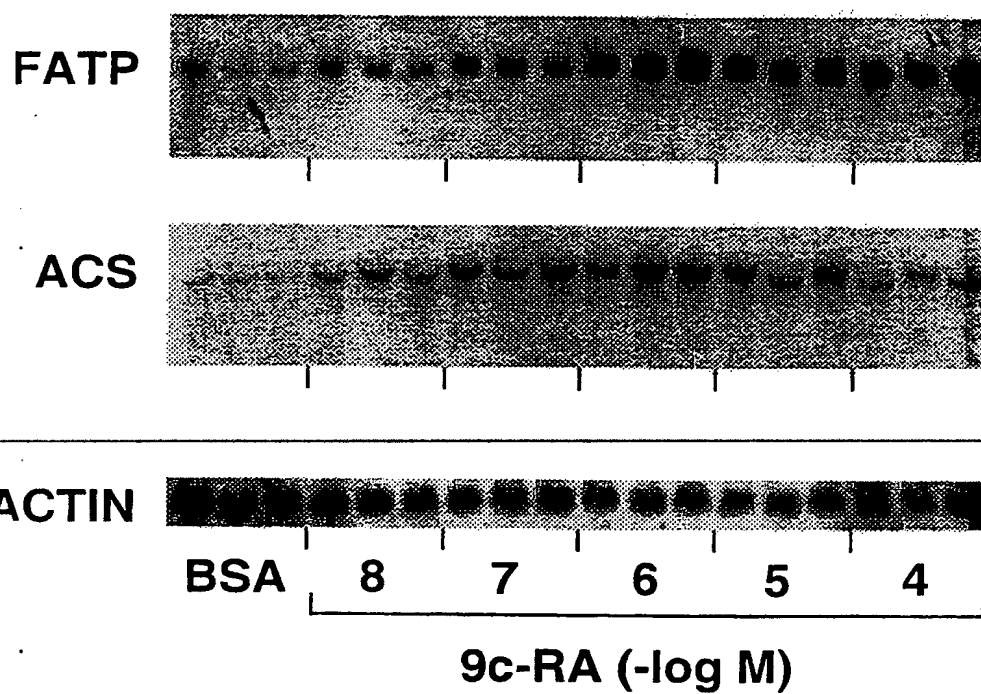


FIG. 10A

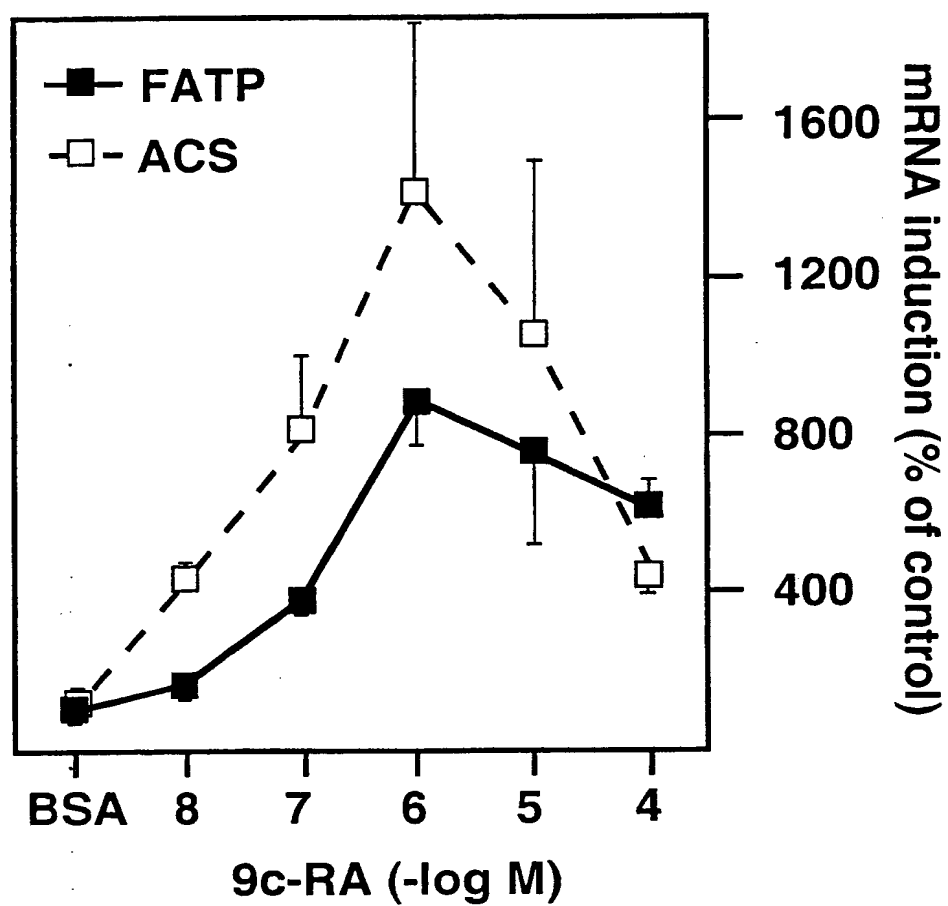


FIG. 10B

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B Hep-G2

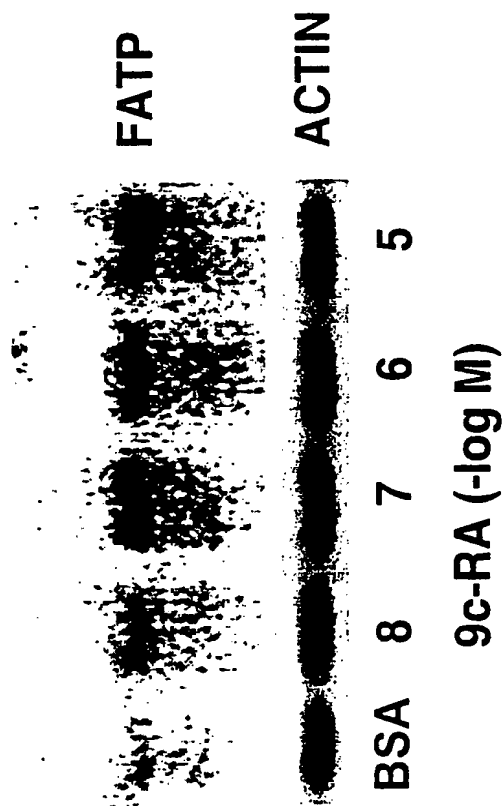
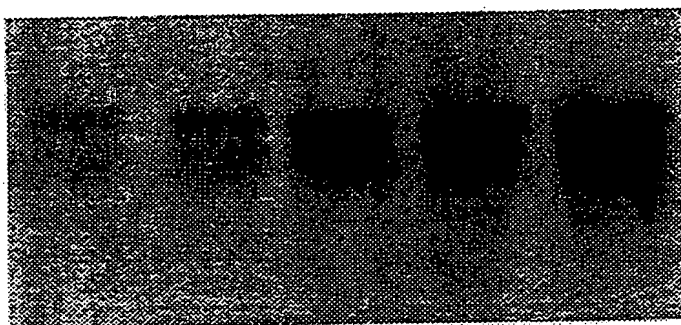
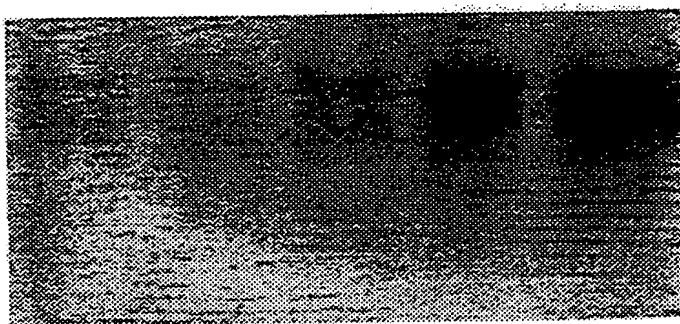
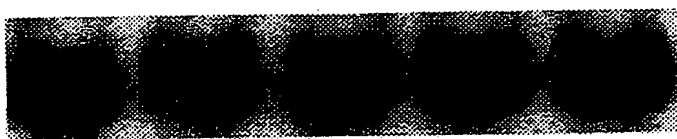


FIG. 10C

A 3T3-L1 Δ **FATP****ACS****ACTIN****BSA 9 8 7 6****9c-RA (-log M)****FIG. 11A****SUBSTITUTE SHEET (RULE 26)**

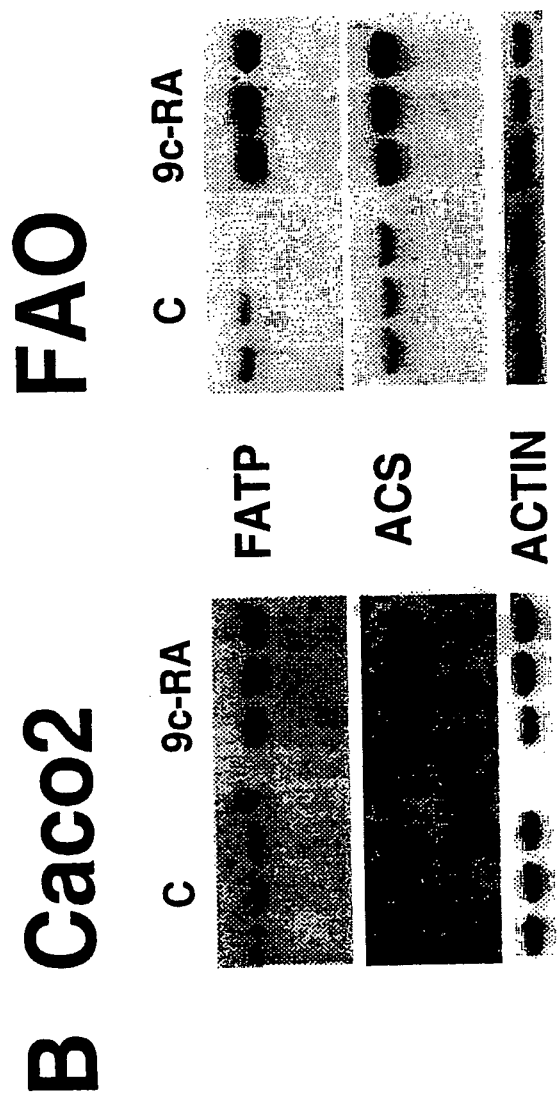


FIG. 11B

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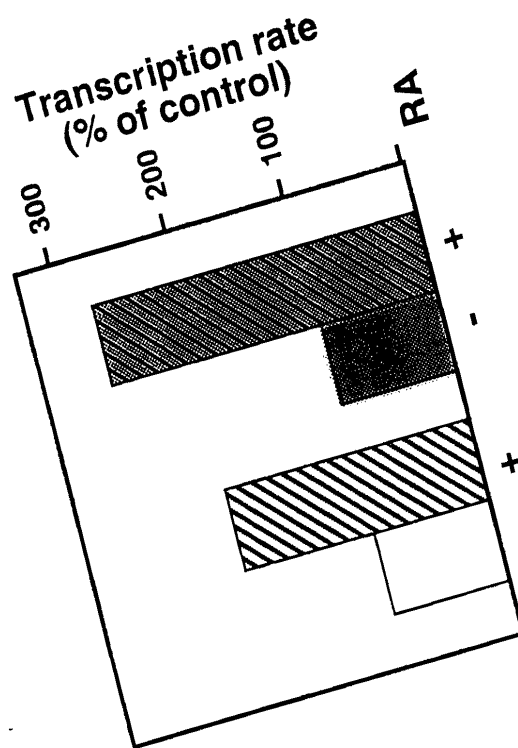
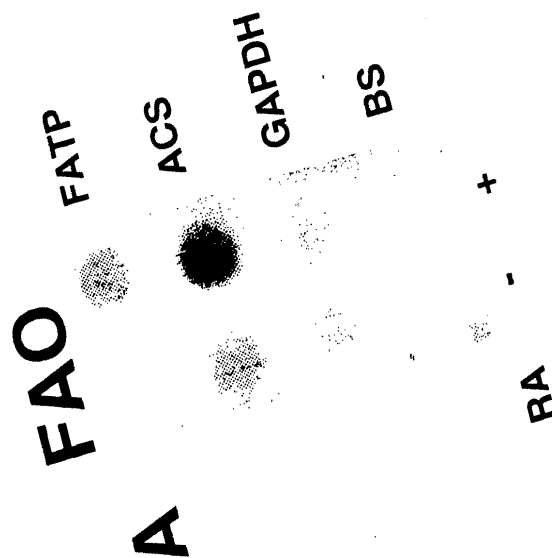


FIG. 12A



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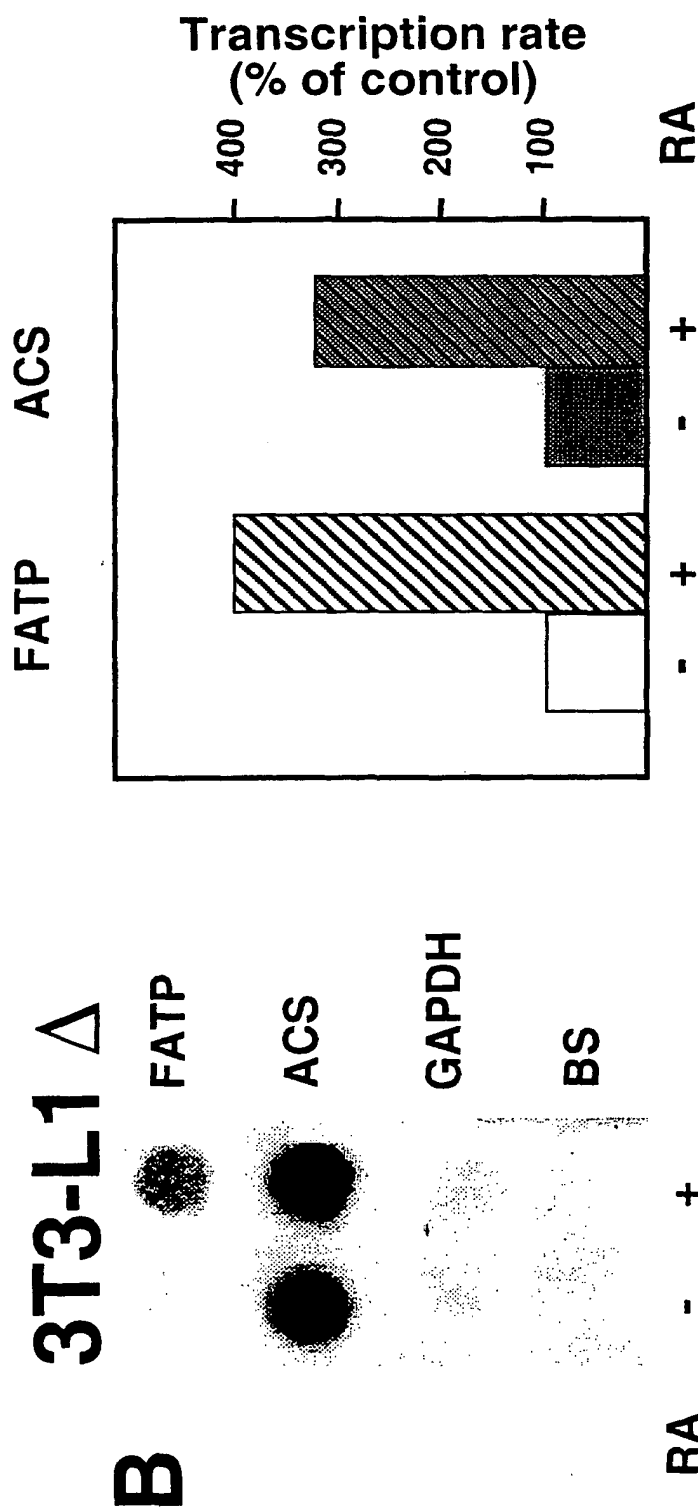


FIG. 12B

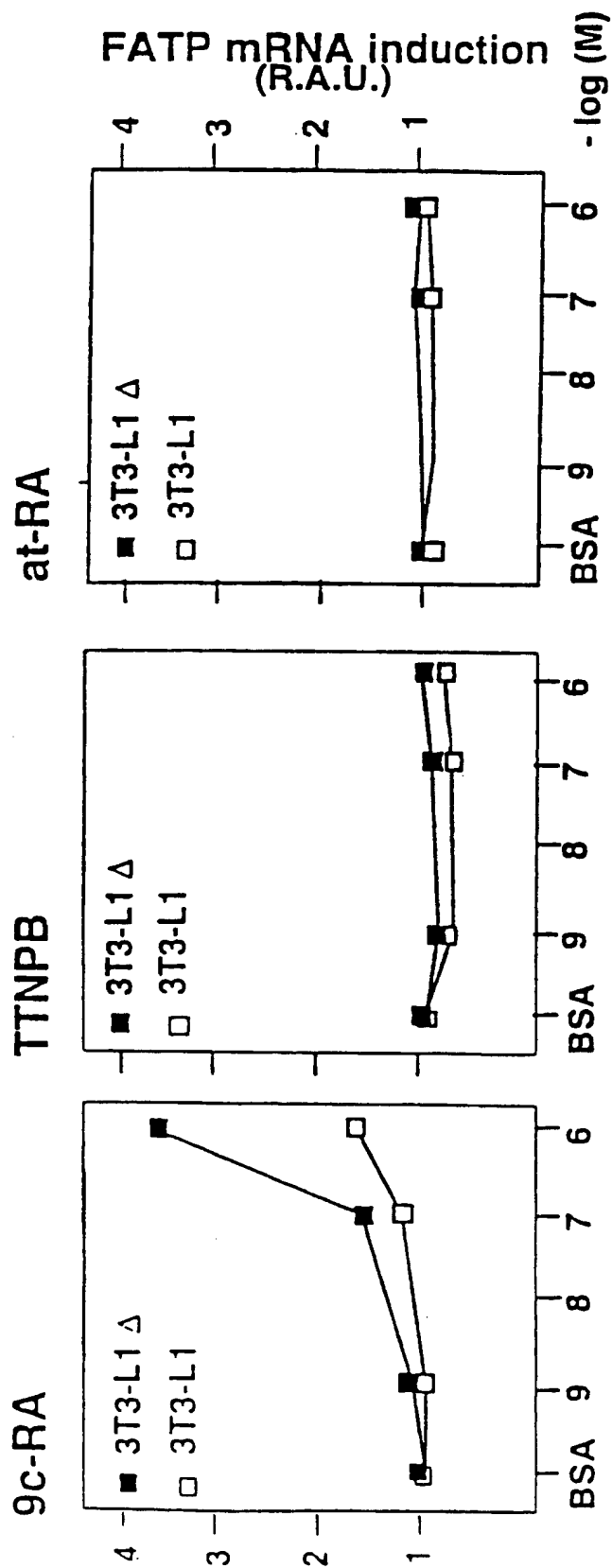


FIG. 13

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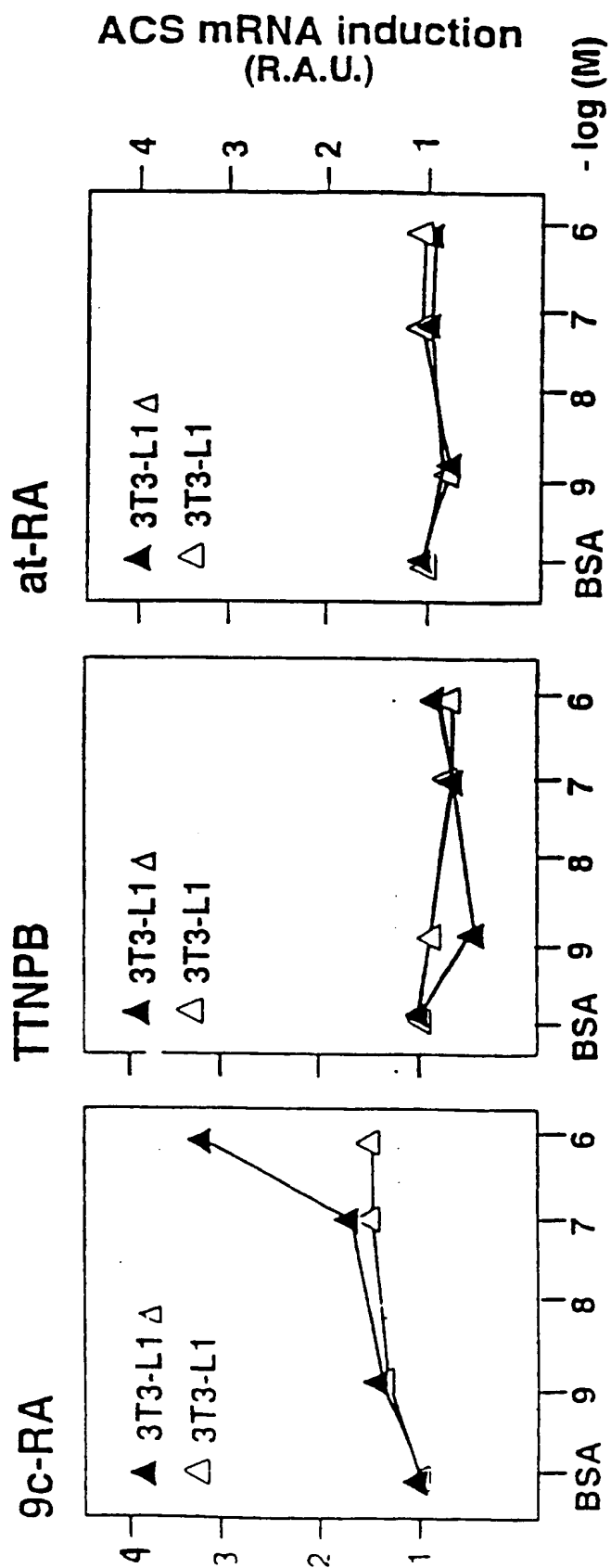


FIG. 14

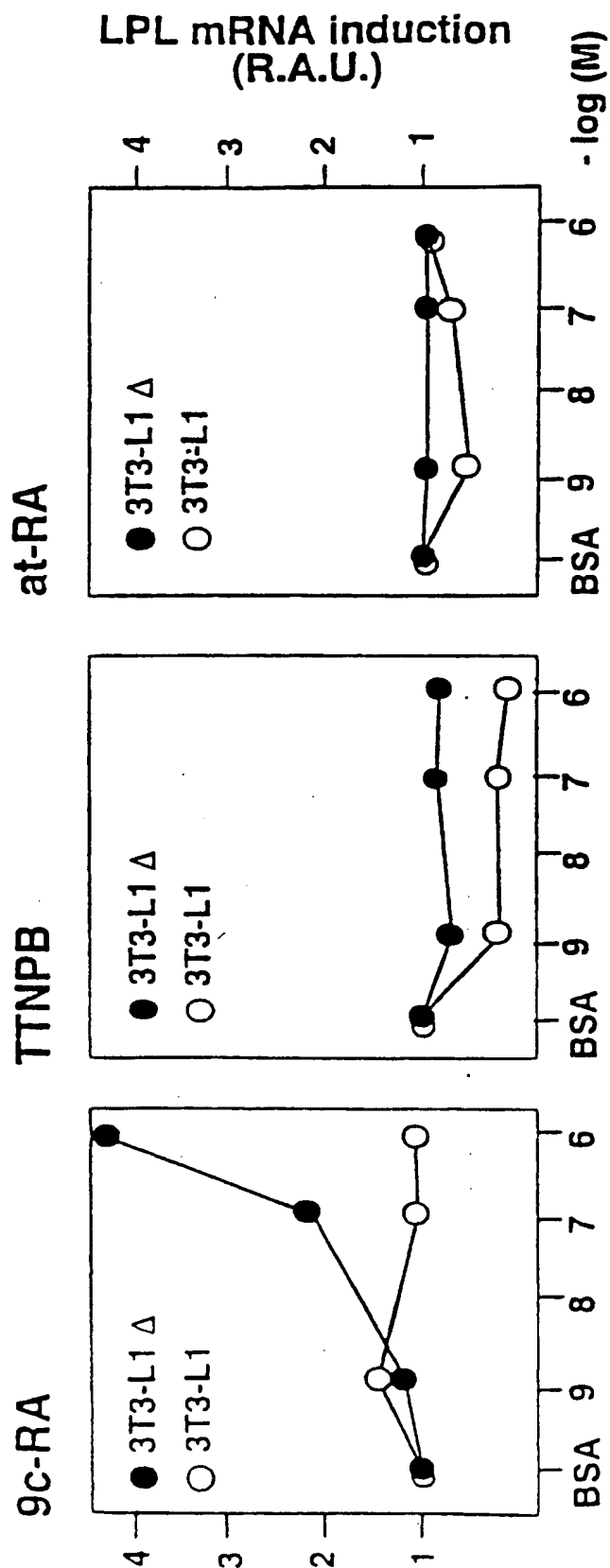


FIG. 15

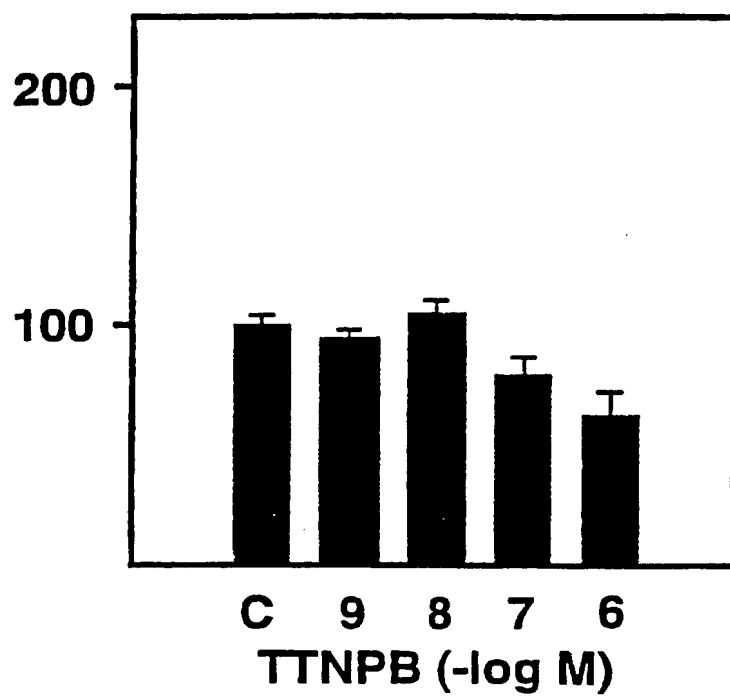


FIG. 16

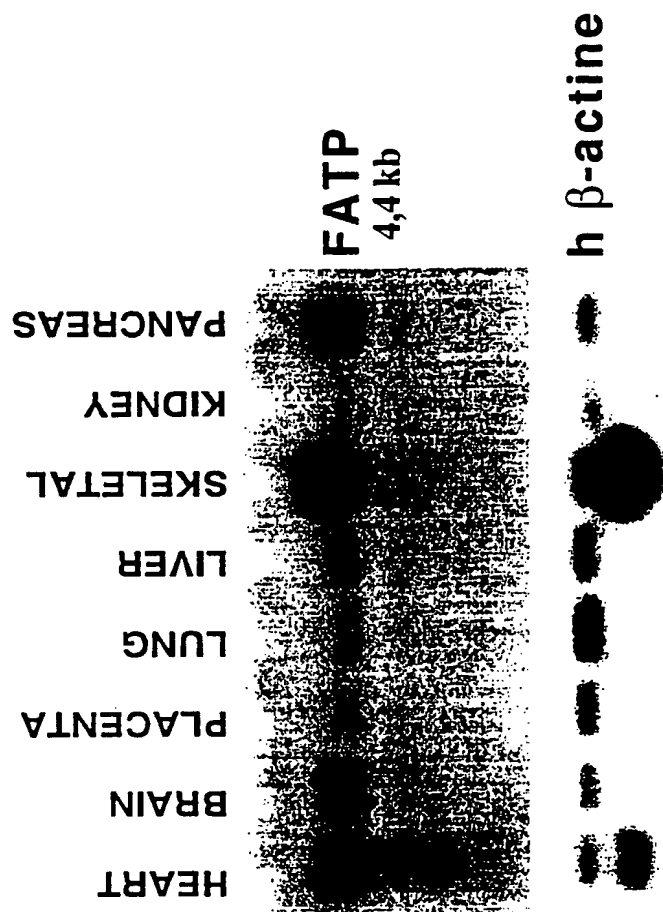


FIG. 17

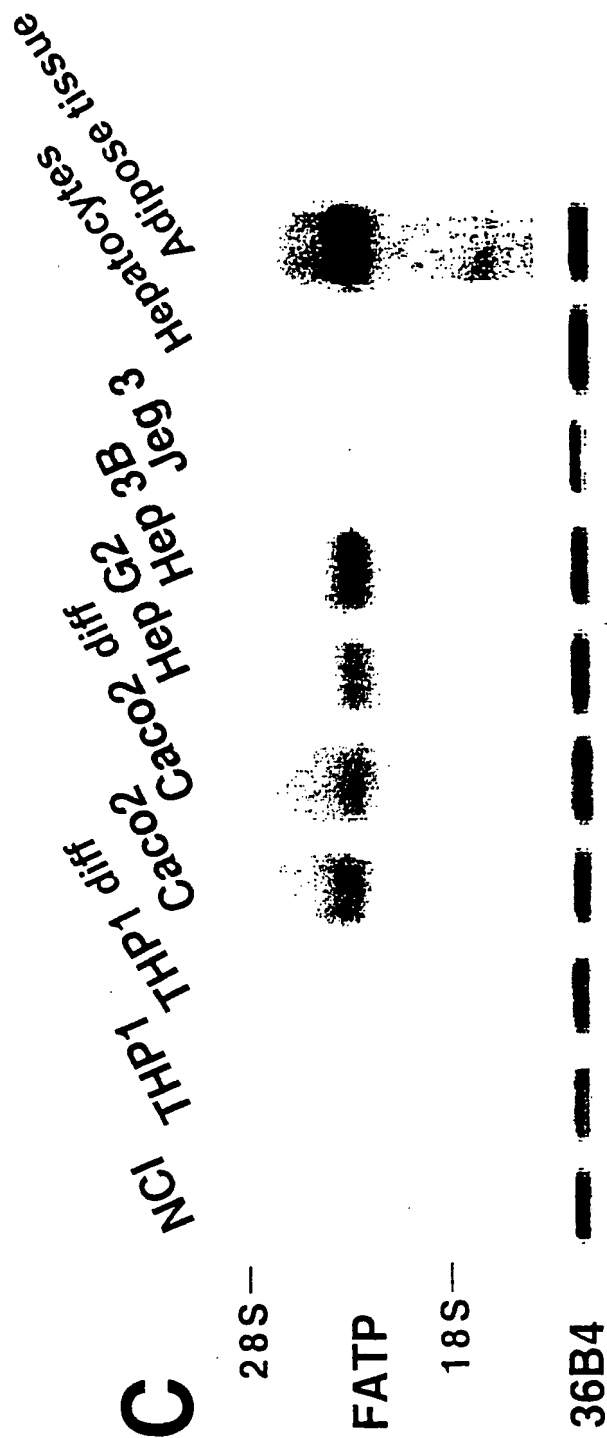


FIG. 18

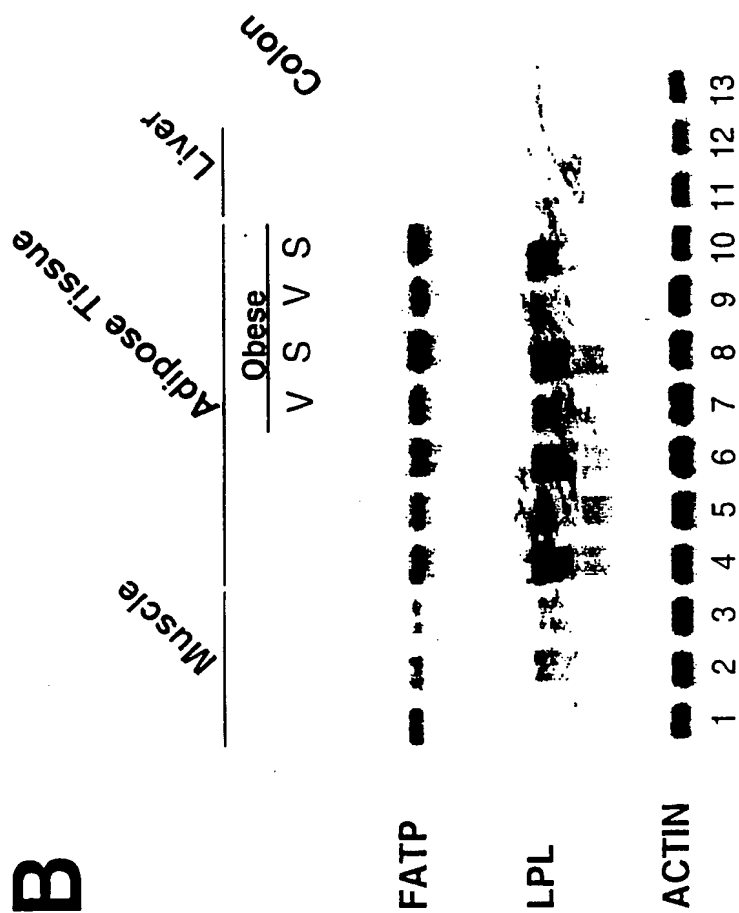


FIG. 19



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : C12N 15/12, C07K 14/47, 16/18</p>	A3	<p>(11) International Publication Number: WO 99/51740</p> <p>(43) International Publication Date: 14 October 1999 (14.10.99)</p>																																										
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>(21) International Application Number: PCT/EP99/02295</p> <p>(22) International Filing Date: 2 April 1999 (02.04.99)</p> <p>(30) Priority Data: 98400823.5 6 April 1998 (06.04.98) EP</p> <p>(71) Applicants (for all designated States except US): JANSSEN PHARMACEUTICA N.V. [BE/BE]; Turnhoutseweg 30, B-2340 Beerse (BE). UNIVERSITY OF WASHINGTON [US/US]; Seattle, WA 98105-4631 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): MARTIN, Geneviève [FR/FR]; 27, rue du Lieutenant Princeteau, F-59800 Lille (FR). NEMOTO, Masami [JP/JP]; Dept. of Internal Medicine (III), Jikei University School of Medicine, 1-17-1, Nishishinbashi, Minato-ku, Tokyo 105 (JP). DEEB, Samir, Sami [US/US]; 846 North East 104th Street, Seattle, WA 98125 (US). AUWERX, Johan [BE/FR]; 60, route d'Hasnon, F-59173 Millonfosse (FR).</p> <p>(74) Agents: ERNEST GUTMANN-YVES PLASSERAUD S.A. et al.; 3, rue Chauveau-Lagarde, F-75008 Paris (FR).</p> </div> <div style="width: 48%;"> <p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p> <p>(88) Date of publication of the international search report: 18 November 1999 (18.11.99)</p> </div> </div>																																												
<p>(54) Title: NUCLEOTIDE SEQUENCE EXPRESSING HUMAN FATTY ACID TRANSPORT PROTEIN AND CORRESPONDING AMINOACID, USE FOR THE REGULATION OF FATTY ACIDS METABOLISM</p>																																												
<table style="margin: auto; border-collapse: collapse;"> <tr> <td></td><td style="text-align: center;">167</td><td></td><td style="text-align: center;">562</td><td></td><td style="text-align: center;">794</td><td style="text-align: center;">724</td><td style="text-align: center;">886</td><td style="text-align: center;">997</td><td></td><td style="text-align: center;">1206</td><td style="text-align: center;">1333</td><td style="text-align: center;">1471</td><td style="text-align: center;">1636</td><td></td><td style="text-align: center;">1783</td> </tr> <tr> <td style="border: 1px solid black; padding: 2px 10px;">1</td> <td style="border: 1px solid black; padding: 2px 10px;">2</td> <td style="border: 1px solid black; padding: 2px 10px;">3</td> <td style="border: 1px solid black; padding: 2px 10px;">4</td> <td style="border: 1px solid black; padding: 2px 10px;">5</td> <td style="border: 1px solid black; padding: 2px 10px;">6</td> <td style="border: 1px solid black; padding: 2px 10px;">7</td> <td style="border: 1px solid black; padding: 2px 10px;">8</td> <td style="border: 1px solid black; padding: 2px 10px;">9</td> <td style="border: 1px solid black; padding: 2px 10px;">10</td> <td style="border: 1px solid black; padding: 2px 10px;">11</td> <td style="border: 1px solid black; padding: 2px 10px;">12</td> </tr> <tr> <td colspan="3" style="text-align: left; padding-left: 10px;">ATG</td> <td colspan="9"></td> <td colspan="2" style="text-align: right; padding-right: 10px;">TGA</td> </tr> </table>				167		562		794	724	886	997		1206	1333	1471	1636		1783	1	2	3	4	5	6	7	8	9	10	11	12	ATG												TGA	
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ATG												TGA																																
<p>(57) Abstract</p> <p>The invention relates to a nucleotide sequence which comprises a sequence involved in the expression of the human Fatty Acid Transport Protein (hFATP) comprising the aminoacid sequence of the Figure.</p>																																												

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/02295

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/47 C07K16/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>MUKHERJEE, R. ET AL.: "Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARgamma2 versus PPARgamma1 and activation with retinoid X receptor agonists and antagonists."</p> <p>JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 12, 21 March 1997 (1997-03-21), pages 8071-8076, XP002081436</p> <p>page 8072, right-hand column, paragraph 2; figures 1,4,5</p> <p style="text-align: center;">--- -/--</p>	<p>1,5,6, 11,16-22</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

5 October 1999

Date of mailing of the international search report

11/10/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Smalt, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/02295

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHAFFER, J.E. ET AL.: "Cloning and structure-function analysis of human heart fatty acid transport protein." CIRCULATION, vol. 96, no. 8, 21 October 1997 (1997-10-21), pages Suppl.-Abstr 2031, XP002082456 abstract ---	5,7,14, 16,18, 21,22,25
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Y	the whole document ---	1-4, 6-11,14, 15,17, 19,20, 23-27
X	SCHAFFER, J.E. ET AL.: "Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein." CELL, vol. 79, 4 November 1994 (1994-11-04), pages 427-36, XP002081438 cited in the application	5,12,16, 18,21, 22,28
Y	the whole document ---	1-4, 6-10,14, 15,17, 19,20, 23-27
Y	DATABASE EMBL - EMBEST11 'Online! Entry HS912118, Acc.No. R48912, 24 May 1995 (1995-05-24) HILLIER, L. ET AL.: "yj69g05.r1 Homo sapiens cDNA clone 154040 5' similar to SP:YBP1_YEAST P38225 HYPOTHETICAL 71.7 KD PROTEIN IN ATP3-RPS18B INTERGENIC." XP002081442 the whole document ---	1-4, 6-11,14, 15,17, 19,20, 23-27
X	DATABASE EMBL - EMBEST2 'Online! Entry AA663208, Acc.No. AA663208, 14 November 1997 (1997-11-14) HILLIER, L. ET AL.: "ab74f07.s1 Stratagene fetal retina 937202 Homo sapiens cDNA clone 852709 3' similar to gb:X07868_rnal PUTATIVE INSULIN-LIKE GROWTH FACTOR II ASSOCIATED (HUMAN)" XP002081443 the whole document ---	5

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/02295

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARTIN, G. ET AL.: "Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthase genes by PPAR-alpha and PPAR-gamma activators." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 45, 7 November 1997 (1997-11-07), pages 28210-7, XP002081439 cited in the application	29-31, 33
Y	the whole document	34, 35
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Y	MUKHERJEE, R. ET AL.: "Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists." NATURE, vol. 386, 27 March 1997 (1997-03-27), pages 407-10, XP002081440	34, 35
A	the whole document	29-33
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	F RGEMAN, N.J. ET AL.: "Disruption of the Saccharomyces cerevisiae homologue to the murine fatty acid transport protein impairs uptake and growth on long-chain fatty acids." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 13, 28 March 1997 (1997-03-28), pages 8531-8, XP002081441 figure 1	
E	---	
	WO 99 36537 A (TARTAGLIA LOUIS A ;WHITEHEAD BIOMEDICAL INST (US); GIMENO RUTH E () 22 July 1999 (1999-07-22) see seq.ID.46 and 47, and the claims. -----	1-38

information on patent family members

PCT/EP 99/02295

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